Rh(D)-BINDING PROTEINS AND MAGNETICALLY ACTIVATED CELL SORTING METHOD FOR PRODUCTION THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

5   This application is entitled to priority pursuant to 35 U.S.C. §119(e) to U.S. provisional patent application 60/081,380, which was filed on April 10, 1998, and is a continuation-in-part of U.S. application 08/884,045, filed June 27, 1997, which application is entitled to priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 60/028,550, filed on October 11,1996.

GOVERNMENT SUPPORT

10   This invention was supported in part by a grant from the U.S. Government (NIH Grant No. P50-HL54516) and the U.S. Government may therefore have certain rights in the invention.

FIELD OF THE INVENTION

15   The field of the invention is generation of binding proteins.

BACKGROUND OF THE INVENTION

The ability to produce monoclonal antibodies has revolutionized diagnostic and therapeutic medicine. Monoclonal antibodies are typically produced by

20   immortalization of antibody-producing mouse lymphocytes thus ensuring an endless supply of cells which produce mouse antibodies. However, for many human applications, it is desirable to produce human antibodies. For example, it is preferable that antibodies which are administered to humans for either diagnostic or therapeutic purposes are human antibodies since administration of human antibodies to a human

25   circumvents potential immune reactions to the administered antibody, which reactions may negate the purpose for which the antibody was administered.

In addition, there exists certain situations where, for diagnostic purposes, it is essential that human antibodies be used because other animals are unable to make antibodies against the antigen to be detected in the diagnostic method. For example, in order to determine the Rh phenotype of human red blood cells (RBCs), human sera that contains anti-Rh antibody must be used since other animals cannot make an antibody capable of detecting the human Rh antigen.

The production of human antibodies *in vitro* by immortalizing human B lymphocytes using Epstein Barr virus (EBV)-mediated transformation or cell fusion has been fraught with technical difficulties due to the relatively low efficiency of both EBV-induced transformation and cell fusion when compared with the murine system. To overcome these problems, processes have been developed for the production of human antibodies using M13 bacteriophage display (Burton et al., 1994, Adv. Immunol. 57:191-280). Essentially, a cDNA library is generated from mRNA obtained from a population of antibody-producing cells. The mRNA encodes rearranged immunoglobulin (Ig) genes and thus, the cDNA encodes the same. Amplified cDNA is cloned into M13 expression vectors creating a library of phage which express human Fab fragments on their surface. Phage which display the antibody of interest are selected by antigen binding and are propagated in bacteria to produce soluble human Fab Ig. Thus, in contrast to conventional monoclonal antibody synthesis, this procedure immortalizes DNA encoding human Ig rather than cells which express human Ig.

There are several difficulties associated with the generation of antibodies using bacteriophage. For example, many proteins cannot be purified in a non-denatured state, in that purification procedures necessarily involve solubilization of protein which may render some proteins permanently denatured with concomitant destruction of antigenic sites present thereon. Such proteins thus cannot be bound to a solid phase and therefore cannot be used to pan for phage bearing antibodies which bind to them. An example of such a protein is the human Rh antigen.

To solve the problem, a method was developed wherein intact RBCs were used as the panning antigen (Siegel et al., 1994, Blood 83:2334-2344). However, it was discovered that since phage are inherently "sticky" and RBCs express a multitude of antigens on the cell surface, a sufficient amount of phage which do not

5   express the appropriate antibody on the surface also adhere to the RBCs, thus rendering the method impractical for isolation of phage which express antibody of desired specificity.

De Kruif et al. (1995, Proc. Natl. Acad. Sci. USA 92:3938-3942) disclose a method of isolating phage encoding antibodies, wherein antibody-expressing

10   phage are incubated with a mixture of antigen-expressing cells and cells which do not express antigen. The antibody-expressing phage bind to the antigen-expressing cells. Following binding with phage, a fluorescently labeled antibody is added specifically to the antigen-expressing cells, which cells are removed from the mixture having antibody-expressing phage bound thereto. The isolation of fluorescently labeled cells

15   is accomplished using the technique of fluorescently-activated cell sorting (FACS), an expensive and time-consuming procedure.

There remains a need for a method of isolating recombinant proteins, preferably antibodies, which is rapid and economical, and which will provide a vast array of protein-binding proteins useful for diagnostic and therapeutic applications in

20   humans.

SUMMARY OF THE INVENTION

The invention relates to an isolated protein having an amino acid sequence comprising a sequence selected from the group consisting of SEQ ID NOs: 1-69 and 139-181. In one embodiment, the isolated protein is an antigen-binding protein.

25   In one aspect, the antigen is human Rh(D) protein. In another embodiment, the binding protein has an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-69 and 139-181. In one aspect, the binding protein is an antibody. In another aspect, the said antibody comprises a heavy chain having an amino acid sequence selected

from the group consisting of SEQ ID NOs: 1-28 and 139-153. In still another aspect, the antibody comprises a light chain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 29-69 and 154-181. In yet another aspect, the antibody comprises a heavy chain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-28 and 139-153 and a light chain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 29-69 and 154-181.

In another embodiment of the isolated binding protein, the binding protein is an antibody fusion protein.

In another embodiment of the isolated protein, the protein is substantially purified.

The invention also includes an isolated DNA encoding the isolated protein of the invention. In one embodiment, the isolated DNA has a nucleotide sequence selected from the group consisting of SEQ ID NOs: 70-138 and 182-224. In another embodiment, the DNA is substantially purified.

The invention also includes an isolated DNA encoding a protein obtained by generating a synthetic DNA library in a virus vector expressing said protein; adding a magnetic label to cells expressing said antigen-bearing moiety; incubating virus expressing said protein with said magnetically labeled cells in the presence of an excess of non-labeled cells which do not express said antigen-bearing moiety to form a mixture, wherein said virus binds to said magnetically labeled cells; isolating virus bound cells from said mixture and obtaining DNA encoding said protein therefrom. In one embodiment, the DNA has a nucleotide sequence selected from the group consisting of SEQ ID NOs: 70-138 and 182-224.

The invention further includes a substantially pure protein obtained by generating a synthetic DNA library in a virus vector expressing said protein; adding a magnetic label to cells expressing said antigen-bearing moiety; incubating virus expressing said protein with said magnetically labeled cells in the presence of an excess of non-labeled cells which do not express said antigen-bearing moiety to form a mixture, wherein said virus binds to said magnetically labeled cells; isolating virus

bound cells from said mixture and isolating said protein therefrom. In one embodiment, the protein has an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-69 and 139-181.

The invention also includes a substantially pure preparation of a protein obtained by expressing said protein from DNA encoding said protein, wherein said DNA is obtained by generating a synthetic DNA library in a virus vector expressing said protein; adding a magnetic label to cells expressing said antigen-bearing moiety; incubating virus expressing said protein with said magnetically labeled cells in the presence of an excess of non-labeled cells which do not express said antigen-bearing moiety to form a mixture, wherein said virus binds to said magnetically labeled cells; isolating virus bound cells from said mixture and obtaining DNA encoding said protein therefrom. In one embodiment, the protein has an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-69 and 139-181.

The invention further relates to a method of isolating a DNA encoding a multi-subunit protein which binds to an antigen-bearing moiety. This method comprises

(a)   generating a phage display library comprising a plurality of virus vectors. A first of the virus vectors comprises a first heterologous DNA encoding a subunit of the protein and expresses the subunit on the surface thereof. A second of the virus vectors comprises a second heterologous DNA encoding a different subunit of the protein and expresses the different subunit on the surface thereof.

(b)   adding a magnetic label to cells bearing the antigen-bearing moiety on their surface.

(c)   incubating the phage display library with the magnetically labeled cells in the presence of an excess of non-labeled cells which do not express the antigen-bearing moiety to form a mixture. The first and second virus vectors thereby bind to the magnetically labeled cells.

(d)   isolating magnetically labeled cells from the mixture. The first and second virus vectors are thereby isolated from the mixture.

(e)   obtaining the first heterologous DNA from the first virus vector.

(f)   ligating at least the portion of the first heterologous DNA encoding the subunit and at least the portion of the second heterologous DNA encoding the different subunit to form a hybrid heterologous DNA.

(g)   generating a hybrid virus vector comprising the hybrid heterologous DNA and expressing the subunit and the different subunit of the protein on the surface thereof.

(h)   adding a magnetic label to cells bearing the antigen-bearing moiety on their surface.

(i)   incubating the hybrid virus vector with the magnetically labeled cells in the presence of an excess of non-labeled cells which do not express the antigen-bearing moiety to form a mixture. The hybrid virus vector thereby binds to the magnetically labeled cells.

(j)   isolating magnetically labeled cells from the mixture. The hybrid virus vector is thereby isolated from the mixture.

(k)   obtaining DNA encoding the protein from the isolated virus vector. The DNA is thereby isolated.

The invention also relates to a method of isolating a multi-subunit protein which binds to an antigen-bearing moiety. This method comprises

(a)   generating a phage display library comprising a plurality of virus vectors. A first of the virus vectors comprises a first heterologous DNA encoding a subunit of the protein and expresses the subunit on the surface thereof. A second of the virus vectors comprises a second heterologous DNA encoding a different subunit of the protein and expresses the different subunit on the surface thereof.

(b)   adding a magnetic label to cells bearing the antigen-bearing moiety on their surface.

(c)   incubating the phage display library with the magnetically labeled cells in the presence of an excess of non-labeled cells which do not express the antigen-bearing moiety to form a mixture. The first and second virus vectors thereby bind to the magnetically labeled cells.

(d)   isolating magnetically labeled cells from the mixture. The first and second virus vectors are thereby isolated from the mixture.

(e)   obtaining the first heterologous DNA from the first virus vector.

(f)   ligating at least the portion of the first heterologous DNA encoding the subunit and at least the portion of the second heterologous DNA encoding the different subunit to form a hybrid heterologous DNA.

(g)   generating a hybrid virus vector comprising the hybrid heterologous DNA and expressing the subunit and the different subunit of the protein on the surface thereof.

(h)   adding a magnetic label to cells bearing the antigen-bearing moiety on their surface.

(i)   incubating the hybrid virus vector with the magnetically labeled cells in the presence of an excess of non-labeled cells which do not express the antigen-bearing moiety to form a mixture. The hybrid virus vector thereby binds to the magnetically labeled cells.

(j)   isolating magnetically labeled cells from the mixture. The hybrid virus vector is thereby isolated from the mixture.

(k)   isolating the protein from the isolated virus vector. The protein is isolated.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagram of a strategy for cell-surface Fab-phage panning using magnetically-activated cell sorting.

Figure 2 is a graph depicting cell-surface biotinylation of human RBCs.

Figure 3 is a series of graphs which validate the antigen-positive, antigen-negative cell separation procedure of the invention.

Figure 4 is an image of a microplate agglutination assay wherein anti-Rh(D) Fab/phage agglutination titer was measured.

Figure 5 is an image of a microplate agglutination assay showing determination of Rh(D) binding epitope for selected anti-Rh(D) Fab/phage clones.

Figure 6 is an image depicting the use of Fab/phage antibodies in a gel card assay.

Figure 7 comprises Figures 7A and 7B. Figure 7A is a dendrogram which depicts the relationship among the anti-Rh(D) heavy chains described herein in Example 2. The 28 unique heavy chain clones are organized by V_H family, V_H germline gene, and VDJ rearrangement. Each heavy chain clone is identified by a numeral preceded by a letter ("B" through "E") which denotes its germline gene. The 28 heavy chains comprised 12 distinct VDJ regions, designated VDJ1 - VDJ12. Clones with identical VDJ joins putatively result from intra-clonal diversity of 12 original B lymphocytes. Figure 7B is an alignment of the CDR3 regions of the anti-Rh(D) heavy chains.

Figure 8 comprises Figures 8A, 8B, and 8C. Figure 8A is an alignment of anti-Rh(D) heavy chains to their nearest germline V, D, and J genes. Also illustrated are the putative intermediate heavy chain sequences (Ca, Cb, Da, Db, Dc). The number of nucleotide differences from a germline V_H is tabulated to the right of each sequence. In general, D segments showed poor homology with known D genes so mutations were not scored in these regions. Replacement mutations are indicated with letters, silent mutations are indicated as "*", identities are indicated as ".", and insertions are indicated as "-". Sequences derived from the 5' V_H primers used in library construction are indicated as ">". Figure 8B is an alignment of the four VH3 genes utilized by anti-Rh(D) heavy chains. Figure 8C is a dendrogram which depicts the relationship among human VH3 family germline genes, and illustrate relatedness of VH3-21, VH3-30. VH3-33, and VH3-30.3 and the surprising restriction in V_H gene usage. The VH3-30.5 gene is present in only certain haplotypes and is identical to VH3-30.

Figure 9 is an ontogenic tree of anti-Rh(D) heavy chains constructed using nucleotide alignment data. Circles represent isolated and sequenced clones, and diamonds represent putative intermediates. The number of nucleotide mutations from its germline V_H gene is indicated in parentheses below the clone name. The distance

along the horizontal axis represents the degree of mutation (including J segments) within the constraints of the diagram.

Figure 10 comprises Figures 10A and 10B. Figure 10A is an alignment of anti-Rh(D) κ light chains to their nearest germline V and J genes, and indicates predominance of DPK-9 usage from the V_κI family. Nomenclature for clones is similar to that for heavy chains but uses the letters "F" through "I". Figure 10B is an alignment of the four V_κ genes utilized by anti-Rh(D) light chains. Symbols are the same as those used in Figure 8A.

Figure 11 comprises Figure 11A and 11B. Figure 11A is an alignment of anti-Rh(D) λ light chains to their nearest germline V and J genes. Figure 11B is an alignment of the 10 V_λ germline genes utilized, and illustrates the use of a diverse set of variable region genes derived from multiple families. However, all of the clones use the identical J_λ gene segment. Nomenclature for the clones is similar to that for heavy chains but uses the letters "J" through "S". Symbols are the same as those used in Figure 8A.

Figure 12, comprising Figures 12A, 12B, and 12C, is a trio of graphs which depict comparisons of variable region gene family usage for anti-Rh(D)-specific clones and randomly-picked, non-Rh(D)-binding clones from original $\gamma_1\kappa$ and $\gamma_1\lambda$ non-selected libraries. Lightly-hatched bars reveal heterogeneity in V_H (Figure 12A), V_κ (Figure 12B), and V_λ (Figure 12C) family representation *before* selection for anti-Rh(D) specificity. Numbers above bars represent absolute number of clones in that group.

Figure 13 depicts the results of determinations of the Rh(D) binding epitope of anti-Rh(D) Fab/phage clones. The five different agglutination patterns obtained from screening all of the 53 Fab/phage clones are illustrated. The particular clones shown in Figure 13 are identified by their unique heavy chain/light chain pairings using the nomenclature defined in Figures 7, 10, and 11. For E1/M3, reactivity with additional Rh(D) variant cells is required to distinguish its specificity

for epD3 from that for epD9. Inclusion of the category IVb cell permits the identification of a new epitope designated "epDX".

Figure 14 is matrix illustrating the genetic composition and epitope specificity of anti-Rh(D) antibodies. The horizontal axis represents the unique γ_1 heavy chains and the vertical axis represents the unique λ and κ light chains (based on amino acid sequence). A shaded pattern at the intersection of a heavy chain/light chain pair indicates the Rh(D) epitope specificity observed for that Fab/phage antibody. A few clones gave mixed patterns of reactivity as described herein. Although heavy chains D1, D15, D16, and D17 differ in nucleotide sequence, these chains have an identical amino acid sequence and thus comprise a single column. Similarly, heavy chains C5 and C8 and λ light chains K1 and K2 encode the same proteins. The pairings of these 28 heavy and 41 light chain nucleotide gene segments, which produced 53 unique Fab transcripts, encoded 43 different Fab proteins, as indicated in the matrix.

Figure 15, comprising Figures 15A, 15B, and 15C, depicts the results of inhibition studies performed using recombinant anti-Rh(D) antibodies. The figures show results of representative experiments demonstrating the mutual inhibition of antibodies directed at two different Rh(D) epitopes (in this example, epD3 and epD6/7, Figures 15A and 15C), but not between an Rh(D) antibody and an unrelated recombinant anti-RBC antibody (an anti-blood group B antibody, Figure 15B). In Figure 15A, Rh(D)-positive RBCs were incubated with soluble Fabs only, phage-displayed Fabs only, or combinations of the two, as indicated. In Figure 15B, Rh(D)-positive RBCs that were blood group B were used. After washing, RBCs were resuspended in anti-M13 antibody and assessed for agglutination induced by phage-displayed Fabs. Soluble Fabs were used "full-strength" while Fab/phage preparations were present in limiting amounts to increase the sensitivity of the inhibition assay, as described herein. In Figure 15C, mutual inhibition of epD3 and epD6/7 anti-Rh(D) antibodies was demonstrated with Rh(D)-positive RBCs, $\gamma_1\kappa$ and $\gamma_1\lambda$ soluble Fabs, and light chain isotype-specific antisera (see text for details). In

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these examples, the anti-epD3 and anti-epD6/7 antibodies were clones E1/M3 and D5/I3, respectively. The anti-blood group B antibody was isolated from an IgG phage display library made from the splenic B cells of a blood group O donor.

Figure 16, comprising Figures 16A, 16B, and 16C, depict models for Rh(D) antigen/antibody binding. A conventional model (depicted in Figure 16A) and a model described herein (depicted in Figure 16B) for Rh(D) antigen/antibody binding predict different combining sites and genetic relationships between antibodies. As depicted in Figure 16C, if antibodies directed at different Rh(D) epitopes are clonally related, then the expressed repertoire will differ between Rh(D)-negative and partial Rh(D) individuals.

DETAILED DESCRIPTION

According to the present invention, there is provided a novel method of isolating DNA encoding a protein and the protein encoded thereby, wherein the protein is preferably an antibody, which protein is capable of specifically binding to an antigen-bearing moiety.

As exemplified herein but not limited thereto, the method comprises generating bacteriophage which encode human antibodies. Specifically in the present invention, anti-Rh(D) RBC Fab/phage antibodies encoded by an M13 filamentous phage library are obtained. The library is generated from antibody-producing cells obtained from a hyperimmunized donor by first obtaining cDNA derived from mRNA expressed in the antibody-producing cells. Ig encoding fragments of the cDNA are obtained using the polymerase chain reaction (PCR) and primers specific for such fragments of DNA. Ig-specific DNA so obtained is cloned into a bacteriophage. Bacteriophage encoding the Ig fragments are panned against a mixture of antigen-positive, biotinylated RBC-target cells pre-coated with streptavidin-conjugated magnetic microbeads and excess non-labeled RBCs. Bacteriophage which express antibodies on the phage surface, which antibodies are specific for the target cell antigen, bind to the labeled cells. These phage are separated from phage which are

bound to non-labeled cells and from phage which are not bound to the cells using a magnetic column. Phage so separated encode and display antibody specific for antigens on the target cells.

To generate a phage antibody library, a cDNA library is first obtained from mRNA which is isolated from cells which express the desired protein to be expressed on the phage surface, *e.g.*, the desired antibody. cDNA copies of the mRNA are produced using reverse transcriptase. cDNA which specifies Ig fragments are obtained by PCR and the resulting DNA is cloned into a suitable bacteriophage vector to generate a bacteriophage DNA library comprising DNA specifying Ig genes. The procedures for making a bacteriophage library comprising heterologous DNA are well known in the art and are described, for example, in Sambrook *et al.* (1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, NY).

A bacteriophage library may also be obtained using cDNA rather than PCR-amplified Ig encoding fragments of cDNA. Generation of a cDNA library is useful for the isolation of proteins which are not antibodies, such as ligands and the like.

Bacteriophage which encode the desired protein, *e.g.*, an antibody, may be engineered such that the protein is displayed on the surface thereof in such a manner that it is available for binding to its corresponding binding protein, *e.g.*, the antigen against which the antibody is directed. Thus, when bacteriophage which express a specific antibody are incubated in the presence of a cell which expresses the corresponding antigen, the bacteriophage will bind to the cell. Bacteriophage which do not express the antibody will not bind to the cell.

For panning of bacteriophage, *i.e.*, selection of phage which express the desired antibody, cells which express the corresponding antigen are labeled with a detectable label such as biotin. Streptavidin-conjugated magnetic beads are then added to the cells. The cells are mixed with an excess of non-labeled cells which do not express the antigen. This cell mixture is then incubated with the phage library, wherein phage which express the antibody bind to cells expressing the antigen. The presence of

the excess non-labeled cells in the mixture serves as a means of removing bacteriophage which do not express the antibody but which might otherwise bind to antigen-expressing cells non-specifically. The details of the experimental procedures for practicing the present invention are provided herein in the experimental detail

5   section.

Antigen-expressing cells having antibody-expressing phage bound thereto are magnetically removed from the mixture. One example of magnetic removal involves pouring the mixture of magnetic and non-magnetic cells into a column in the selective presence or absence of a magnetic field surrounding the column.

10   Alternatively, magnetic cells may be separated from non-magnetic cells in solution by simply holding a magnet against the side of a test tube and attracting the cells to the inner wall and then carefully removing the non-magnetic cells from the solution.

Thus, the method of the invention involves a procedure for enriching a population of recombinant phage for those expressing specific phage-displayed ligands

15   derived from natural or synthetic phage DNA libraries by simultaneously performing negative and positive selection against a mixture of magnetically-labeled receptor-positive particles (*i.e.*, cells) and non-labeled receptor-negative particles.

The terms "bacteriophage" and "phage" are used interchangeably herein and refer to viruses which infect bacteria. By the use of the terms "bacteriophage

20   library" or "phage library" as used herein, is meant a population of bacterial viruses comprising heterologous DNA, *i.e.*, DNA which is not naturally encoded by the bacterial virus.

The term "virus vector" includes a virus into which heterologous DNA has been inserted. The virus vector may be a bacteriophage or may be a eukaryotic

25   virus.

By the term "target cell" as used herein, is meant a cell which expresses an antigen against which the desired antibody is sought.

By the term "panning" or "panned" as used herein, is meant the process of selecting phage which encode the desired antibody.

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By the term "Fab/phage" as used herein, is meant a phage particle which expresses the Fab portion of an antibody.

By the term "scFv/phage" are used herein, is meant a phage particle which expresses the Fv portion of an antibody as a single chain.

5   By "excess non-labeled cells" is meant an amount of non-labeled cells which exceeds the number of labeled cells. Preferably, the ratio of labeled cells to non-labeled cells is about 1:2. More preferably, the ratio of labeled cells to non-labeled cells is greater than about 1:4. Even more preferably, the ratio of labeled cells to non-labeled cells is greater than about 1:10.

10   While the method of the invention as exemplified herein describes the generation of phage which encode the Fab portion of an antibody molecule, the method should not be construed to be limited solely to the generation of phage encoding Fab antibodies. Rather, phage which encode single chain antibodies (scFV/phage antibody libraries) are also included in the invention. Fab molecules comprise the entire Ig light

15   chain, that is, they comprise both the variable and constant region of the light chain, but include only the variable region and first constant region domain (CH1) of the heavy chain. Single chain antibody molecules comprise a single chain of protein comprising the Ig Fv fragment. An Ig Fv fragment includes only the variable regions of the heavy and light chains of the antibody, having no constant region contained therein. Phage

20   libraries comprising scFV DNA may be generated following the procedures described in Marks *et al.*, 1991, *J. Mol. Biol.* **222**:581-597. Panning of phage so generated for the isolation of a desired antibody is conducted as described herein for phage libraries comprising Fab DNA.

The invention should also be construed to include synthetic phage

25   display libraries in which the heavy and light chain variable regions may be synthesized such that they include nearly all possible specificities. Therefore, antibody-displaying libraries can be "natural" or "synthetic" (Barbas, 1995, *Nature Medicine* **1**:837-839; de Kruif *et al.* 1995, *J. Mol. Biol.***248**:97-105). Antibody-displaying libraries comprising "natural" antibodies are generated as described in the

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experimental example section. Antibody-displaying libraries comprising "synthetic" antibodies are generated following the procedure described in Barbas (1995, *supra*) and the references cited therein.

The method of the invention should be further construed to include generation of phage display libraries comprising phage other than M13 as exemplified herein. Other bacteriophage, such as lambda phage, may also be useful in the method of the invention. Lambda phage display libraries have been generated which display peptides encoded by heterologous DNA on their surface (Sternberg *et al.*, 1995, *Proc. Natl. Acad. Sci. USA* **92**:1609-1613). Moreover, it is contemplated that the method of the invention may be extended to include viruses other than bacteriophage, such as eukaryotic viruses. In fact, eukaryotic viruses may be generated which encode genes suitable for delivery to a mammal and which encode and display an antibody capable of targeting a specific cell type or tissue into which the gene is to be delivered. For example, retroviral vectors have been generated which display functional antibody fragments (Russell *et al.*, 1993, *Nucl. Acids Res.* **21**:1081-1085).

The red blood cell antibodies to which antibodies may be generated include, but are not limited to, Rh antigens, including Rh(D), Rh(C), Rh(c), Rh(E), Rh(e), and other non-Rh antigens, including red blood cell antigens in the Kell, Duffy, Lutheran and Kidd blood groups.

Thus, the method of the invention is not limited solely to the isolation of DNA encoding anti-Rh(D) antibodies, but rather may be used for the isolation of DNA encoding antibodies directed against any RBC antigen or other cell antigen, such as, but not limited to, tumor-specific antigen, bacterial antigens, and the like. The method of the invention is also useful for typing platelets by generating phage antibodies specific for a number of clinically important platelet antigens, notably, Pl^{A1}/Pl^{A2}, Bak^a/Bak^b, Pen^A/Pen^B, and the like.

The invention is further useful for typing donor white blood cells for HLA antigens for the purposes of matching donors and recipients for potential

transplant matching in the case of both solid (for example, kidney, heart, liver, lung) and non-solid (for example, bone marrow) organ or tissue transplanting.

To detect binding of phage expressing antibody directed against one of these non-red blood cell antigens, the non-red blood cells may be agglutinated or

5   trapped following the procedures described herein for agglutination or trapping of red blood cells. Prior to agglutination or trapping, the cells may be rendered "visible" by staining or other labeling technique in order that agglutination or trapping is apparent to the naked eye or scanner.

The method of the invention is most useful for the generation of a

10   protein which binds to an antigen-bearing moiety, where the antigen-bearing moiety is not easily purified in soluble form. Thus, the antigen-bearing moiety includes antigens which are associated with other structures, usually membranes in the cell such as cell membranes or cell organelle membranes.

In accordance with the present invention, the antigen-bearing moiety

15   may be a protein, a lipid, a carbohydrate or a nucleic acid, or it may be a complex of at least two of a protein, a lipid, a carbohydrate and a nucleic acid, it being appreciated that many antigen-bearing moieties in cells are not comprised of one of these components alone. Preferably, the antigen-bearing moiety is a membrane bound protein, such as an antigen or a receptor protein. However, when the antigen-bearing

20   moiety is a carbohydrate, it may be a carbohydrate expressed on a glycolipid, for example, a P blood group antigen or other antigen.

By the term "antigen-bearing moiety" as used herein, is meant a molecule to which an antibody binds.

By the term "antigen-binding protein" as used herein, is meant a

25   polypeptide molecule, such a an antibody, a fragment thereof or an antibody fusion protein, which is capable of specifically binding to another molecule.

By the term "antibody fusion protein" as used herein, is meant a polypeptide molecule having an amino acid sequence which comprises the amino acid

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sequence of a portion of an antigen-binding protein. The portion of the antigen-binding protein may, for example, be an entire antibody or a fragment thereof.

The method of the invention is also useful for the generation of autoimmune antibodies such as those involved in autoimmune hemolytic anemia (AIHA) (Siegel *et al.*, 1994, *Structural analysis of red cell autoantibodies*, Garratty (*ed.*) *Immunobiology of Transfusion Medicine*, Dekker, New York, New York). Autoimmune antibodies that are directed against cell antigens which are cell surface membrane associated or cell organelle membrane associated may be isolated using the technology described herein. Autoimmune diseases and their associated antigens to which antibodies may be isolated include, but are not limited to the following: Myasthenia gravis (acetylcholine receptor; neurons), chronic inflammatory demyelinating polyneuropathy (myelin; neurons), autoimmune thyroid disease (thyroid stimulating hormone receptor; thyroid cells), primary biliary cirrhosis (mitochondrial autoantigens; liver mitochondria), idiopathic thrombocytopenic purpura (platelet membrane integrins; platelets), pemphigus vulgaris (epidermal antigens; epidermis), and Goodpasture's syndrome (basement membrane antigens; kidney or lung cells).

In fact, the method of the invention is useful for the isolation of DNA clones encoding any antibody directed against an antigen expressed on a cell, which cell can be labeled with a magnetic label and which cell can be obtained in sufficient quantities in an non-labeled form so as to provide an excess of non-labeled cells as required in the assay.

Further, the method of the invention is not limited to the isolation of DNA encoding antibodies but rather may also be used for the isolation of DNA encoding other peptides or proteins having specificity for cell proteins, such as, for example, but not limited to, ligands which bind cell receptor proteins, peptide hormones, and the like.

The invention should also not be construed as being limited to the use of biotin as the cell-labeling agent. Other labels may be used provided their addition to a cell does not disturb the structural integrity of any surface proteins expressed thereon

and provided such labels permit the addition of a paramagnetic microbead or other magnetic substance thereto. Other such labels include, but are not limited to, cell surface proteins or carbohydrates which can be directly derivitized with magnetic beads that possess activated amine, carboxyl, or thiol groups. In addition, dyes such as fluorescein or rhodamine may also be covalently attached to cells in a manner similar to biotin and magnetic beads coated with anti-dye antibodies may be attached thereto.

The invention also includes a screening method which may be used to isolate a DNA encoding a multi-subunit protein which binds to an antigen-bearing moiety or, alternately, to isolate the multi-subunit protein itself. The multi-subunit protein may, for example, be an antibody or another immunoglobulin. It is well known that antibodies and other immunoglobulins comprise multiple subunits, often designated heavy and light chains.

According to this screening method, a phage display library is generated, either as described herein or using other generally known or hereafter-developed methods. The library comprises a plurality of virus vectors, including a first virus vector which comprises a first heterologous DNA encoding a subunit of the protein. The first virus vector expresses the subunit on its surface, either by itself or in association with one or more other subunits of the protein. The library also comprises a second virus vector which comprises a second heterologous DNA encoding a different subunit of the protein. The second virus vector expresses the different subunit on its surface, either by itself or in association with one or more other subunits of the protein. A magnetic label is added to cells bearing the antigen-bearing moiety on their surface, and the labeled cells are incubated with the phage display library in the presence of an excess of non-labeled cells which do not express the antigen-bearing moiety. The first and second virus vectors bind to the magnetically labeled cells, owing to interaction(s) between the antigen and the subunits of the protein expressed on the surface of the vectors.

After incubating the phage display library with the mixture of cells, magnetically labeled cells are isolated from the mixture. First and second virus vectors

bound to the magnetically labeled cells are thereby also isolated from the mixture. The virus vectors are separated from the magnetically labeled cells (e.g. by culturing the cells in a manner in which the virus vectors are produced in the culture supernatant), and heterologous DNA is obtained from virus vectors that adhered to the magnetically labeled cells. The DNA may optionally be purified at this stage. DNA isolated from the virus vectors that adhered to the magnetically labeled cells includes the first heterologous DNA and the second heterologous DNA.

At least the portion of the first heterologous DNA encoding the subunit is ligated to at least the portion of the second heterologous DNA encoding the different subunit to form a hybrid heterologous DNA. For this purpose, it is advantageous that the virus vector be constructed in such a way that the portion of the first heterologous DNA encoding the subunit, the portion of the second heterologous DNA encoding the different subunit, or both, are flanked or surrounded by defined restriction endonuclease cleavage sites. In such constructs, the portion of the first heterologous DNA encoding the subunit may be removed, for example, by treating the first heterologous DNA with restriction endonucleases which specifically cleave the specific sites. This portion may then be ligated, for example either directly or after ligating a linker DNA thereto, to all or a portion of the second heterologous DNA to generate the hybrid heterologous DNA.

The hybrid heterologous DNA is then used to generate a hybrid virus vector comprising the hybrid heterologous DNA. The hybrid virus vector expresses the subunit and the different subunit of the protein on its surface. For example, if the first heterologous DNA encodes an antibody light chain and the second heterologous DNA encodes an antibody heavy chain, then the hybrid virus vector may express an antibody comprising equal numbers of heavy and light chains on its surface.

The hybrid virus vector is then incubated with the mixture of magnetically labeled cells having the antigen-bearing moiety on their surface and non-magnetically labeled cells which do not have the antigen-bearing moiety on their surface. Owing to interactions between the antigen and the subunits of the protein

expressed on the surface of the hybrid virus vector, the hybrid virus vector binds with the magnetically labeled cells, and may therefore be isolated from the mixture of cells by isolating magnetically labeled cells from the mixture.

As described herein, hybrid virus vector particles are isolated from the magnetically labeled cells. The isolated hybrid virus vectors may be used as a source for obtaining either the multi-subunit protein or the hybrid heterologous DNA (which encodes the subunits of the protein), using standard methods.

The invention includes proteins and DNA encoding the same which are generated using the methods described herein. To isolate DNA encoding an antibody, for example, DNA is extracted from antibody expressing phage obtained according to the methods of the invention. Such extraction techniques are well known in the art and are described, for example, in Sambrook *et al.* (*supra*).

The invention includes a number of isolated or substantially purified DNAs encoding antigen-binding proteins, such as Rh(D)-binding proteins. For example, a DNA having a nucleotide sequence comprising at least one of SEQ ID NOs: 70-138 and 182-224, as described herein, is included. The isolated or substantially purified nucleic acid may have a nucleotide sequence selected from the group consisting of SEQ ID NOs: 70-138 and 182-224.

An "isolated DNA", as used herein, refers to a DNA sequence, segment, or fragment which has been purified from the sequences which flank it in a naturally occurring state, *e.g.*, a DNA fragment which has been removed from the sequences which are normally adjacent to the fragment, *e.g.*, the sequences adjacent to the fragment in a genome in which it naturally occurs. The term also applies to DNA which has been substantially purified from other components which naturally accompany the DNA, *e.g.*, RNA or DNA or proteins which naturally accompany it in the cell.

The invention also includes a number of isolated or substantially purified proteins, such as Rh(D)-binding proteins. For example, a protein having an amino acid sequence comprising at least one of SEQ ID NOs: 1-69 and 139-181, as

described herein, is included. The isolated or substantially purified protein may have an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-69 and 139-181. The protein may be an antigen-binding protein, such as an antibody which comprises a heavy chain having an amino acid sequence selected from the group

5   consisting of SEQ ID NOs: 1-28 and 139-153, a light chain having an amino acid sequence selected from the group consisting of SEQ ID NOs 29-69 and 154-181, or both. The protein may also be, for example, an antibody fusion protein.

An "isolated protein" as used herein, means a protein or polypeptide which has been separated from components which naturally accompany it in a cell.

10   Typically, a protein or polypeptide is isolated when at least 10%, more preferably at least 20%, more preferably at least 50% of the total material (by volume, by wet or dry weight, or by mole percent or mole fraction) in a sample is the protein or polypeptide of interest.

The invention should also be construed to include DNAs which are

15   substantially homologous to the DNA isolated according to the method of the invention. Preferably, DNA which is substantially homologous is about 50% homologous, more preferably about 70% homologous, even more preferably about 80% homologous and most preferably about 90% homologous to DNA obtained using the method of the invention.

20   "Homologous" as used herein, refers to the subunit sequence similarity between two polymeric molecules, e.g., between two nucleic acid molecules, e.g., two DNA molecules or two RNA molecules, or between two polypeptide molecules. When a subunit position in both of the two molecules is occupied by the same monomeric subunit, e.g., if a position in each of two DNA molecules is occupied by adenine, then

25   they are homologous at that position. The homology between two sequences is a direct function of the number of matching or homologous positions, e.g., if half (e.g., five positions in a polymer ten subunits in length) of the positions in two compound sequences are homologous then the two sequences are 50% homologous, if 90% of the

positions, *e.g.*, 9 of 10, are matched or homologous, the two sequences share 90% homology. By way of example, the DNA sequences
3' ATTGCC 5' and 3' TATGCG 5' share 50% homology.

To obtain a substantially pure preparation of a protein comprising, for example, an antibody, generated using the methods of the invention, the protein may be extracted from the surface of the phage on which it is expressed. The procedures for such extraction are well known to those in the art of protein purification. Alternatively, a substantially pure preparation of a protein comprising, for example, an antibody, may be obtained by cloning an isolated DNA encoding the antibody into an expression vector and expressing the protein therefrom. Protein so expressed may be obtained using ordinary protein purification procedures well known in the art.

As used herein, the term "substantially pure" describes a compound, *e.g.*, a protein or polypeptide which has been separated from components which naturally accompany it. Typically, a compound is substantially pure when at least 10%, more preferably at least 20%, more preferably at least 50%, more preferably at least 60%, more preferably at least 75%, more preferably at least 90%, and most preferably at least 99% of the total material (by volume, by wet or dry weight, or by mole percent or mole fraction) in a sample is the compound of interest. Purity can be measured by any appropriate method, *e.g.*, in the case of polypeptides by column chromatography, gel electrophoresis or HPLC analysis. A compound, *e.g.*, a protein, is also substantially purified when it is essentially free of naturally associated components or when it is separated from the native contaminants which accompany it in its natural state.

As used herein, amino acids are represented by the full name thereof, by the three letter code corresponding thereto, or by the one-letter code corresponding thereto, as indicated in the following table:

Full Name	Three-Letter Code	One-Letter Code
Aspartic Acid	Asp	D
Glutamic Acid	Glu	E

	Lysine	Lys	K
	Arginine	Arg	R
	Histidine	His	H
	Tyrosine	Tyr	Y
5	Cysteine	Cys	C
	Asparagine	Asn	N
	Glutamine	Gln	Q
	Serine	Ser	S
	Threonine	Thr	T
10	Glycine	Gly	G
	Alanine	Ala	A
	Valine	Val	V
	Leucine	Leu	L
	Isoleucine	Ile	I
15	Methionine	Met	M
	Proline	Pro	P
	Phenylalanine	Phe	F
	Tryptophan	Trp	W

The present invention also provides for analogs of proteins or peptides

20 obtained according to the methods of the invention. Analogs can differ from naturally occurring proteins or peptides by conservative amino acid sequence differences or by modifications which do not affect sequence, or by both.

For example, conservative amino acid changes may be made, which although they alter the primary sequence of the protein or peptide, do not normally alter

25 its function. Conservative amino acid substitutions typically include substitutions within the following groups:

glycine, alanine;

valine, isoleucine, leucine;

aspartic acid, glutamic acid;

asparagine, glutamine;

serine, threonine;

lysine, arginine;

phenylalanine, tyrosine.

5 Modifications (which do not normally alter primary sequence) include *in vivo*, or *in vitro* chemical derivatization of polypeptides, *e.g.*, acetylation, or carboxylation. Also included are modifications of glycosylation, *e.g.*, those made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing or in further processing steps; *e.g.*, by exposing the polypeptide to enzymes which affect

10 glycosylation, *e.g.*, mammalian glycosylating or deglycosylating enzymes. Also embraced are sequences which have phosphorylated amino acid residues, *e.g.*, phosphotyrosine, phosphoserine, or phosphothreonine.

Also included in the invention are polypeptides which have been modified using ordinary molecular biological techniques so as to improve their

15 resistance to proteolytic degradation or to optimize solubility properties. Analogs of such polypeptides include those containing residues other than naturally occurring L-amino acids, *e.g.*, D-amino acids or non-naturally occurring synthetic amino acids. The peptides of the invention are not limited to products of any of the specific exemplary processes listed herein.

20 In addition to substantially full length polypeptides, the present invention provides for active fragments of the polypeptides. A specific polypeptide is considered to be active if it binds to an antigen-bearing moiety, for example, if a fragment of an antibody binds to its corresponding antigen in the same manner as the full length protein.

25 As used herein, the term "fragment," as applied to a polypeptide, will ordinarily be at least about fifty contiguous amino acids, typically at least about one hundred contiguous amino acids, more typically at least about two hundred continuous amino acids and usually at least about three hundred contiguous amino acids in length.

The invention is further described in detail by reference to the following experimental examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Thus, the invention should in no way be construed as being limited to the following examples, but rather, should be construed to encompass any and all variations which become evident as a result of the teaching provided herein.

Example 1
Isolation of Cell Surface-Specific Human Monoclonal Antibodies
Using Phage Display and Magnetically-Activated Cell Sorting

The experiments described in this Example provide procedures and results for the isolation and production of anti-Rh(D) red blood cell antibodies using Fab/phage display.

A method is described in Figure 1 for the isolation of filamentous phage-displayed human monoclonal antibodies specific for non-purifiable cell surface expressed molecules. To optimize the capture of antigen-specific phage and minimize the binding of irrelevant phage antibodies, a simultaneous positive and negative selection strategy was employed. Cells bearing the antigen of interest are pre-coated with magnetic beads and are diluted into an excess of unmodified antigen-negative cells. Following incubation of the cell admixture with a Fab/phage library, the antigen positive cell population is retrieved using magnetically-activated cell sorting, and antigen-specific Fab/phage are eluted and propagated in bacterial culture. When this protocol was used with magnetically-labeled (Rh(D)-positive and excess non-labeled Rh(D)-negative human red blood cells and a Fab/phage library constructed from human peripheral blood lymphocytes, dozens of unique, clinically useful $_{\gamma 1}\kappa$ and $_{\gamma 1}\lambda$ anti-Rh(D) antibodies were isolated from a single alloimmunized individual.

The cell-surface selection method of the present invention is readily adaptable for use in other systems, such as for the identification of putative tumor-specific antigens, and provides a rapid (less than one month), high yield approach for

isolating self-replicative antibody reagents directed at novel or conformationally-dependent cell-surface epitopes.

Creation of Fab/phage display libraries

Separate $_{\gamma 1}\kappa$ and $_{\gamma 1}\lambda$ phage libraries were constructed from 2×10^7 mononuclear cells derived from the peripheral blood from an Rh(D)-negative individual previously hyperimmunized with Rh(D)-positive red blood cells (RBCs). The phagemid vector pComb3 (Barbas, 1991, Proc. Natl. Acad. Sci. USA 88:7978-7982) was used to create the libraries utilizing previously published methods (Barbas et al., 1991, Combinatorial immunoglobulin libraries on the surface of phage (Phabs): Rapid selection of antigen-specific Fabs. Methods: A Companion to Methods in Enzymology 2:119-124; Siegel et al., 1994, Blood 83:2334-2344).

Briefly, cDNA was prepared from the mRNA of the donor cells and heavy chain and light chain immunoglobulin (Ig) cDNA segments were amplified using the polymerase chain reaction (PCR) and the battery of human Ig primers described by Kang et al. (1991, "Combinatorial Immunoglobulin Libraries on the Surface of Phage (Phabs): Rapid Selection of Antigen-Specific Fabs. Methods: A Companion to Methods" in Enzymology 2:111-118) supplemented by those of Silverman et al. (1995, J. Clin. Invest. 96:417-426). The heavy and light chain PCR products were cloned into pComb3 and electroporated into *E. coli*. Upon co-infection with VCSM13 helper phage (Stratagene, La Jolla, CA), Ig DNA was packaged into filamentous phage particles which express human Fab molecules fused to the gene III bacteriophage coat protein.

Panning Fab phage display libraries for anti-Rh(D) clones

Rh(D)-positive RBCs were cell-surfaced biotinylated by incubating cells at a hematocrit of 10% with 500 µg/ml sulfo-NHS-LC-biotin (Pierce Chemical, Rockford, IL) for 40 minutes at room temperature (RT). Following 5 washes with phosphate-buffered saline (PBS), 8×10^6 biotinylated Rh(D)-positive RBCs were incubated with 10 µl of streptavidin-coated paramagnetic microbeads (MACS Streptavidin Microbeads, Mitenyi Biotec, Sunnyvale, CA) for 1 hour at RT in a total

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volume of 100 µl PBS. Non-reacted beads were removed by washing and then the magnetic bead-coated, Rh(D)-positive RBCs were mixed with a 10-fold excess (8 x 10^7) of the Rh(D)-negative (unmodified) RBCs and ~3 x 10^{11} colony-forming units (cfu) of either the $_{\gamma 1}\kappa$ and $_{\gamma 1}\lambda$ Fab/phage libraries (prepared as described above) in a final volume of 40 µl PBS containing 2% non-fat dry milk (MPBS, Carnation, Nestle Food Products, Glendale, CA).

Following a 2 hour incubation at 37°C, the RBC/phage suspension was loaded at a flow rate of 10 µl/minute onto a MiniMACS magnetic type MS column (Mitenyi Biotec, Sunnyvale, CA) that was pre-equilibrated with 2% MPBS. This loading step was performed without a magnetic field around the column so as to prevent magnetic bead-coated RBCs from instantly adhering to the very top of the column, clogging it, and causing the trapping of Rh(D)negative non-biotinylated RBCs. Loading the RBC/phage incubation mixture in the absence of a magnetic field causes the antigen-negative and antigen-positive RBCs to distribute evenly throughout the column without running off since the excluded volume of the column is slightly greater than 40 µl. Once loaded, the column was placed in a magnetic field (MiniMACS magnetic separation unit, Mitenyi Biotec, Sunnyvale, CA) for 2 minutes to allow the Rh(D)-positive RBCs to adhere, and a series of 500 µl washes were performed with ice-cold MPBS followed by a final wash with PBS. A total of 3 washes were performed for the first 2 rounds of panning and a total of 6 washes were performed for all subsequent pannings. For each panning, the first wash was carried out at a flow rate of 10 µl/minute during which time the bulk of Rh(D)-negative RBCs washed off the column. All subsequent washes were performed at 200 µl/minute. Following the last wash, the column was removed from the magnetic field and the bead-coated/phage-coated Rh(D)-positive RBCs were flushed off the column with 500 µl PBS using the plunger from a 5 cc syringe (Becton-Dickinson, Franklin Lakes, NJ).

The RBCs were immediately centrifuged for 5 seconds at 13,000 x g and were then resuspended in 200 µl of 76 mM citrate, pH 2.4, to denature the Rh(D) antigen and elute bound phage. Following a 10 minute incubation period at RT with

intermittent vortexing, the phage eluate and cellular debris were neutralized with 18 μl 2 M Tris base and were added to 10 ml of O.D.=1.0 XL1-Blue strain of *E. coli* (Stratagene, La Jolla, CA) grown in super broth (SB) (Barbas *et al.*, 1991, *supra*) supplemented with 10 μg/ml tetracycline. After incubation for 15 minutes at RT,

5   during which time the phage library enriched for Rh(D) binders was allowed to infect the bacterial culture, 10 ml of pre-warmed, 37°C SB containing 40 μg/ml carbenicillin/10 μg/ml tetracycline was added to give final antibiotic concentrations of 20 μg/ml and 10 μg/ml, respectively. A small aliquot of culture (~100 μl) was immediately removed and titered on Luria broth/carbenicillin plates to determine the

10   number of phage contained in the total eluate. The balance of the culture was shaken at 37°C for 1 hour at 300 RPM. Additional antibiotics, additional SB, and VCSM13 helper phage were subsequently added and the culture was grown overnight at 30°C as described (Siegel et al., 1994, *supra*).

        Phagemid particles were purified from the culture supernatant by

15   polyethylene glycol 8000 (PEG) precipitation (Barbas et al., 1991, *supra*), resuspended in 1% bovine serum albumin (BSA)/PBS, and dialyzed overnight to remove residual PEG that may lyse RBCs during subsequent rounds of panning. Thus, the resultant phage preparation serves as the input for the next round of panning. The $_{\gamma 1}\kappa$ and $_{\gamma 1}\lambda$ phage libraries were panned separately to prevent any bias in light chain isotype

20   replication possibly introduced by bacterial amplification.

        Screening polyclonal Fab/phage libraries and individual phage colonies for anti-Rh(D) reactivity

        The specificity of Fab/phage for the Rh(D) antigen was assessed using anti-M13 antibody as a bridging antibody to induce agglutination between RBCs that

25   have bound anti-Rh(D) Fab/phage. One hundred μl aliquots of polyclonal Fab/phage from rounds of panning, or monoclonal Fab/phage derived from individual Fab/phage eluate clones, were incubated with 50 μl of a 3% suspension of RBCs of defined phenotype (*i.e.*, Rh(D)-negative or -positive).

Following 1 hour incubation at 37°C, the RBCS were washed 3 times with 2 ml cold PBS to remove unbound Fab/phage. The resultant RBC pellets were resuspended in 100 μl of a 10 μg/ml solution of sheep anti-M13 antibody (5-Prime 3-Prime, Boulder, CO) and transferred to the round-bottomed wells of a 96-well

5   microtiter plate. Plates were left undisturbed (~2 hours) and were then read. Wells having a negative reaction exhibit sharp ~2 millimeter diameter RBC spots whereas in wells having positive reactions, *i.e.*, agglutination, the RBCs in agglutinated wells form a thin carpet coating the entire floor of the well.

For hemagglutination assays utilizing mini-column gel cards (ID-Micro-

10   Typing System, Ortho Diagnostics, Raritan, NJ) (Lapierre et al., 1990, Transfusion 30:109-113), 25 μl of Fab/phage clones were mixed with 50 μl aliquots of RBCs (0.8% suspensions in Micro Typing System buffer, Ortho Diagnostics). The mixtures were placed in the reservoirs above the mini-columns which contain dextran-acrylamide beads previously suspended in 100 μl/ml anti-M13 antibody. After incubation at 37°C,

15   the gel cards were centrifuged at 70 x g for 10 minutes and were read.

Miscellaneous methods

Preparation of fluorescently-labeled RBCs for flow cytometry was performed as described herein and samples were analyzed using a FACScan microfluorimeter equipped with Lysis II (Ver 1.1) software (Becton-Dickinson,

20   Mountain View, CA). Plasmid DNA was prepared from bacterial clones (Qiawell Plus, Qiagen, Chatsworth, CA). Double-stranded DNA was sequenced using light chain or heavy chain Ig constant region reverse primers or unique pComb3 vector primers that anneal 5-prime to the respective Ig chain (Barbas et al., 1991, *supra*; Roben et al., 1995, J. Immunol. 154:6437-6445) and automated fluorescence sequencing (Applied

25   Biosystems, Foster City, CA). Sequences were analyzed using MacVector Version 5.0 sequencing software (Oxford Molecular Group, Oxford, UK) and the Tomlinson database of Ig germline genes (Tomlinson et al., 1996, V Base Sequence Directory. MRC Center for Protein Engineering, Cambridge, UK).

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Experimental design for cell incubation and separation protocols

The experimental conditions described above for panning Fab/phage libraries for anti-RBC-reactive phage were determined after performing a series of initial studies aimed at optimizing the cell separation process and ultimate yield of

5   antigen-specific Fab/phage. The main parameters investigated included:

Biotinylation Conditions were sought that would biotinylate the RBC surface in a manner such that a sufficient number of streptavidin-coated magnetic beads would bind to the cells causing the RBCs to be retained by a magnetic column. In this case, over-biotinylation that might destroy the antigenicity of the Rh(D) antigen or

10   might make the cells non-specifically absorb antibody is to be avoided. To address this issue, Rh(D)-positive/Kell-negative RBCs (Kell being a RBC antigen; (Walker, ed. 1993, In: Technical Manual, 11th Ed., Bethesda, MD, American Association of Blood Banks) were incubated with a range of sulfo-NHS-LC-biotin concentrations and the degree of biotinylation was assessed by flow cytometry utilizing fluorescein-

15   conjugated streptavidin.

To assess the degree of cell-surface biotinylation, 5 µl aliquots of 3% suspensions of Rh(D)-positive/Kell-negative RBCs biotinylated at varying biotin reagent concentrations were incubated with 200 µl of a 1/100 dilution of FITC-streptavidin (Jackson ImmunoResearch, Bar Harbor, Maine) for 30 min at 4°C (Figure

20   2). The mixture was washed with phosphate buffered saline (PBS) and analyzed by flow microfluorimetry (–□–). Aliquots of cells were also analyzed for retention of Rh(D)-antigenicity (–Δ–) (i.e., specific staining) or for lack of non-specific staining (–O–) by incubating the cells with 100 µl of either anti-Rh(D) or anti-Kell typing sera, respectively, washing the cells and then staining them with a 1/100 dilution of FITC-

25   goat anti-human IgG (Jackson ImmunoResearch).

A linear, non-saturating response was observed (Figure 2). Retention of Rh(D) antigenicity was assessed using anti-Rh(D) typing serum and was found to be unaffected by the derivatization of cell-surface proteins with biotin at all biotin

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concentrations tested (Figure 2). Furthermore, the Kell-negative RBCs did not non-specifically adsorb anti-Kell antibodies.

Each biotinylated RBC sample was then incubated with an excess of streptavidin-coated magnetic microbeads and applied to a magnetic separation column. It was determined that as many as 10^8 RBCs could be retained by the column for RBC samples biotinylated with greater than or equal to 500 µg/ml biotin reagent. Since the actual RBC/phage panning experiments were designed to use only ~10^7 Rh(D)-positive cells (see below), RBC biotinylation at 500 µg/ml was determined to be sufficient.

Concentration of Rh(D)-positive and Rh(D)-negative RBCs in incubation mixture

Prior to performing Fab/phage panning experiments, the ability of the magnetically-activated cell separation technique to separate Rh(D)-positive and Rh(D)-negative cells was assessed using anti-Rh(D) typing serum and flow cytometry (Figure 3). Streptavidin-microbead coated, biotinylated Rh(D)-positive RBCs (8 x 10^6 cells) were mixed with a 10-fold excess of Rh(D)-negative non-coated RBCs (8 x 10^7 cells) in a 40 µl volume of PBS containing 2% non-fat dry milk (MPBS) and the mixture was applied to a MiniMACS column. The column was washed and the bound cells were eluted as described herein. Aliquots of RBCs contained in the original admixture (panel a), the column wash (panel b), and the column eluate (panel c) were stained with anti-Rh(D) typing serum and FITC-goat anti-human IgG as described in Figure 2. The flow cytograms show that although ~90% of the cells in the column load were Rh(D)-negative (panel a), nearly all of them washed off of the column (panel b), yielding a column eluate that was almost entirely Rh(D)-positive cells (panel c). Since only ~6% of the final eluate comprise Rh(D)-negative cells (panel c), and Rh(D)-negative cells were initially present in a 10-fold excess to Rh(D)-positive cells, only ~0.6% of the initial antigen-negative immunosorbent cells contaminated the final antigen-positive preparation. This efficiency of the cell separation was deemed adequate for subsequent panning experiments with Fab/phage.

In the above-described experiment, to avoid clogging the magnetic separation column, it was necessary to load the column in the absence of a magnetic field. This necessitated a reaction volume of less than or equal to 40 μl so that none of the material would run off the column. On theoretical grounds (Kretzschmar *et al.*, 1995, Anal. Biochem. 224:413-419), one can calculate the appropriate concentration of cells required in a 40 μl volume to capture greater than 50% of Fab/phage specific for a given cell surface antigen. Such a calculation is a function of the number of antigen sites per cell and the dissociation constant (K_D) of the bound Fab/phage. Using a value of ~100,000 Rh(D) antigen sites per RBC (phenotype "-D-/-D-") (Mollison et al., 1993, In: Blood Transfusion in Clinical Medicine, Oxford, Blackwell Scientific Publications) and the desired Fab/phage affinity in the $K_D = 10^{-8}$ to 10^{-9} M range, then 8×10^6 Rh(D)-positive RBCs in a 40 μl reaction volume would be required. Given this number of Rh(D)-positive cells, a 10-fold excess of Rh(D)-negative RBCs was found to be the maximum amount of antigen-negative cells that could be effectively separated from antigen-positive RBCs by the magnetic column (Figure 3).

Construction and panning of Fab/phage libraries

$_{\gamma1}\kappa$ and $_{\gamma1}\lambda$ phage libraries were prepared as described herein and were found to contain 7×10^7 and 3×10^8 independent transformants, respectively. Table 1 tabulates the panning results for the libraries.

An RBC agglutination assay utilizing anti-M13 secondary antibody as bridging antibody was used to detect anti-Rh(D) Fab/phage activity in the panned polyclonal libraries and the individual randomly-picked Fab/phage clones (Figure 4). The results shown are a representative example of the assay depicting negative reactivity to Rh(D)-negative RBCs and strongly positive reactivity to Rh(D)-positive RBCs for the $_{\gamma1}\kappa$ library (panning #2) out to a dilution of 1/2048.

In the case of the $_{\gamma1}\kappa$ library, significant enrichment for binding phage appears to occur after only one round of panning, whereas significant enrichment for the $_{\gamma1}\lambda$ library occurs during the second round. This is reflected by both the sharp increase in the percent of phage bound during a given round of panning as well as the

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ability of the polyclonal $_{\gamma1}\kappa$ and $_{\gamma1}\lambda$ Fab/phage libraries to agglutinate Rh(D)-positive RBCs after 1 and 2 rounds of panning, respectively (Table 1, Figure 4).

Monoclonal Fab/phage were prepared from randomly-picked individual bacterial colonies obtained during each round of panning. It was apparent that by the third round of panning, all clones have anti-Rh(D) specificity (Table 1). To confirm that these Fab/phage have anti-Rh(D) specificity and are not binding to other unrelated antigens that may coincidentally be present on the particular Rh(D)-positive RBC and absent on the particular Rh(D)-negative RBC used in the agglutination assays, clones were screened against a panel of 11 Rh(D)-negative and-positive RBCs of varying blood group specificities to verify their anti-Rh(D) specificity (Walker, 1993, *supra*).

Clonal analysis at the genetic level

To investigate the genetic diversity among the randomly picked anti-Rh(D) clones, plasmid DNA was prepared from each of the clones and the corresponding heavy and light chain Ig nucleotide sequences were identified. In Table 2 there is listed a number of attributes for each clone including the name of the most closely-related germline heavy or light chain Ig gene. More detailed analysis at the nucleotide level revealed that among all of the anti-Rh(D) binding clones, there were a large number of unique heavy and light chain DNA sequences (Table 3). Because of the random shuffling of heavy and light chain gene segments which occurs during the creation of a Fab/phage display library (Barbas et al., 1991, *supra*), it is evident that these heavy chains and light chains combined to form nearly 50 different anti-Rh(D) antibodies.

A detailed multiple alignment analysis of the predicted amino acid sequences revealed a total of twenty-five unique heavy chain, eighteen unique kappa light chain and twenty-three unique lambda light chain proteins. Due to the combinatorial effect during library construction, these heavy and light chain gene segments paired to produce fifty unique Fab antibodies ($20_{\gamma1\kappa}$ and $30_{\gamma1\lambda}$). Of interest, all twenty five unique heavy chains and nearly all of the eighteen unique kappa light chains were derived from only 5 $V_H III$ or four $V\kappa I$ germline genes, respectively, while

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the lambda light chains were derived from a more diverse set of germline genes. Analysis of heavy and light chain nucleotide sequences from over sixty negative clones from the non-panned libraries were performed to verify the heterogeneity in variable region family representation before selection. Clones representing V_H families I

5   (13%), III (36%), IV (31%), V(15%) and VI (5%); Vκ families I (43%), II (14%), III (29%) and IV (14%); and Vγ families I (48%), II (4%), III (9%), IV (4%), V (9%), VI (17%) and VII (9%) were present.

Clonal analysis at the protein level

To investigate the diversity in fine specificity (Rh(D) antigen epitope

10   specificity) among the anti-Rh(D) clones, agglutination experiments were performed with selected clones and with sets of rare Rh(D)-positive RBCs which were obtained from individuals whose RBCs produce Rh(D) antigen lacking certain epitopes. Examining the pattern of agglutination of a particular anti-Rh(D) antibody with such sets of mutant RBCs enables the identification of the specific epitope on Rh(D) to

15   which the antibody is directed (Mollison et al., 1993, *supra*). A representative example of such an experiment is shown in Figure 5 and the Rh(D) epitopes for selected anti-Rh(D) Fab/phage clones are tabulated in Table 2.

Agglutination experiments were performed with anti-Rh(D)-negative RBCs (rr), Rh(D)-positive RBCs (R_2R_2), and "partial" Rh(D)-positive RBCs (mosaics

20   IIIa, IVa, Va, VI, VII). The results shown are a representative example of the assay for 5 randomly-picked anti-Rh(D) Fab/phage clones (Figure 5).

TABLE 1a. $\gamma_1\kappa$FAB/PHAGE LIBRARY PANNING RESULTS

PANNING[1]	ϕINPUT (CFUs)[2]	ϕOUTPUT (CFUs)[3]	%BOUND (x 10^{-4})[4]	ENRICHMENT[5]	AGGLUT TITER[6]	BINDERS/ TOTAL(%)[7]
0					0	0/16 (0)
1	2.94×10^{11}	6.04×10^{5}	2.1		1/16	0/16 (0)
2	2.15×10^{11}	1.68×10^{7}	78.3	38.0 x	1/2048	15/15 (100)
3	1.72×10^{11}	1.44×10^{8}	840.0	10.7 x	1/2048	12/12 (100)

TABLE 1b. $\gamma_1\lambda$FAB/PHAGE LIBRARY PANNING RESULTS

PANNING[1]	ϕINPUT (CFUs)[2]	ϕOUTPUT (CFUs)[3]	%BOUND (x 10^{-4})[4]	ENRICHMENT[5]	AGGLUT TITER[6]	BINDERS/ TOTAL(%)[7]
0					0	0/16 (0)
1	2.28×10^{11}	3.48×10^{5}	1.5		0	
2	5.51×10^{11}	1.34×10^{6}	2.4	1.6 x	1/128	32/36 (89)
3	3.93×10^{11}	3.86×10^{8}	980.0	404.0 x	1/512	24/24 (100)
4	2.87×10^{11}	3.08×10^{8}	1100.0	1.1 x	1/1024	

[1] panning round, where "0" represents the initial, non-panned Fab/phage library

[2] number of colony-forming units (CFUs) of phage (ϕ) incubated with Rh(D)-positive/-negative RBC admixture

[3] total number of CFUs of ϕ contained in eluate

[4] (ϕ output/ϕinput) x 100

[5] fold increase in % bound from compared to previous round of panning

[6] agglutination titer; see text and Figure 4

[7] number of Rh(D)-binding Fab/phage clones per total number of clones screened from panning round; see Table 2 for details

TABLE 2a. ANALYSIS OF $_{\gamma 1}\kappa$FAB/PHAGE CLONES						
CLONE[1]	AGGLU[2]	VH FAM[3]	VH GENE[4]	Vκ FAM[5]	Vκ GENE[6]	D EPITOPE[7]
KPO-1	neg	3	DP-47/V3-23	4	DPK24/VklVKlobeck	
KPO-2	neg	3	DP-31/V3-9P	3	DPK22/A27	
KPO-3	neg	3	DP-58/hv3d1EG	4	DPK24/VklVKlobeck	
KPO-4	neg	4	3d279d+	--	no light chain	
KPO-5	neg	3	DP-29/12-2	1	LFVK431	
KPO-6	neg	4	DP-79/4d154	1	DPK9/012	
KPO-7	neg	3	V3-48/hv3d1	4	DPK24/VklVKlobeck	
KPO-8	neg	4	DP-70/4d68	2	DPK18/A17	
KPO-9	neg	1	DP-14/V1-18	1	DPK9/012	
KPO-10	neg	4	DP-70/4d68	1	DPK9/012	
KPO-11	neg	5	DP-73/V5-51	1	DPK9/012	
KPO-12	neg	3	DP-54/V3-7	2	DPK18/A17	
KPO-13	neg	3	V3-48/hv3d1	1	Vb'	
KPO-14	neg	6	DP-74/VH-VI	1	DPK6/Vb"	
KPO-15	neg	3	DP-46/3d216	3	Vg/38K	
KPO-16	neg	6	DP-74/VH-VI	1	DPK9/012	
KP1-1	neg	4	V71-4+	3	DPK22/A27	
KP1-2	neg	4	3d279d+	1	DPK8/Vd+	
KP1-3	neg	1	4M28	1	DPK9/012	
KP1-4	neg	4	DP-79/4d154	3	Vg/38K	
KP1-5	neg	3	DP-38/9-1	3	DPK22/A27	
KP1-6	neg	4	DP-70/4d68	1	L12a/PCRdil6-5	
KP1-7	neg	5	DP-73/V5-51	2	DPK15/A19	

TABLE 2a. ANALYSIS OF $_{\gamma 1}\kappa$FAB/PHAGE CLONES						
CLONE[1]	AGGLU[2]	VH FAM[3]	VH GENE[4]	Vκ FAM[5]	Vκ GENE[6]	D EPITOPE[7]
KP1-8	neg	4	DP-70/4d68	3	DPK22/A27	
KP1-9	neg	--	no heavy chain	--	no light chain	
KP1-10	neg	--	no heavy chain	3	DPK22/A27	
KP1-11	neg	1	DP-15/V1-8+	1	DPK9/012	
KP1-12	neg	3	b28e	--	no light chain	
KP1-13	neg	3	DP-47/V3-23	4	DPK24/VklVKlobeck	
KP1-14	neg	1	DP-31/V3-9P	3	DPK21/humkv328h5	
KP1-15	neg	1	DP-7/21-2	4	DPK24/VklVKlobeck	
KP1-16	neg	5	DP-73/V51	3	DPK22/A27	
KP2-1	pos	3	DP-50/hv3019b9	1	DPK9/012	epD6/7
KP2-2	pos	3	DP-50/hv3019b9	1	DPK9/012	epD6/7
KP2-3	pos	3	DP-50/hv3019b9	1	DPK9/012	epD6/7
KP2-4	pos	3	b28m	1	DPK9/012	epD2
KP2-5	pos	3	b28m	1	DPK9/012	epD1
KP2-6	pos	3	DP-50/hv3019b9	1	DPK9/012	epD6/7
KP2-7	pos	3	DP-50/hv3019b9	1	DPK9/012	epD5
KP2-8	pos	3	DP-50/hv3019b9	1	DPK9/012	
KP2-9	pos	3	DP-50/hv3019b9	1	DPK9/012	epD2
KP2-10	pos	3	DP-50/hv3019b9	1	DPK9/012	epD2
KP2-11	pos	3	DP-50/hv3019b9	1	DPK9/012	epD2
KP2-12	pos	3	DP-50/hv3019b9	1	DPK9/012	epD1
KP2-13	pos	3	DP-50/hv3019b9	1	DPK9/012	epD6/7

CLONE[1]	AGGLU[2]	VH FAM[3]	VH GENE[4]	Vκ FAM[5]	Vκ GENE[6]	D EPITOPE[7]
KP2-14	pos	3	DP-50/hv3019b9	2	DPK15/A19	epD2
KP2-15	pos	3	DP-50/hv3019b9	1	DPK9/O12	epD6/7
KP3-1	pos	3	DP-50/hv3019b9	1	DPK9/O12	
KP3-2	pos	3	DP-50/hv3019b9	1	DPK9/O12	epD6/7
KP3-3	pos	3	DP-50/hv3019b9	1	DPK9/O12	
KP3-4	pos	3	DP-49/1.9III	1	DPK9/O12	epD5
KP3-5	pos	3	DP-50/hv3019b9	1	DPK9/O12	
KP3-6	pos	3	DP-50/hv3019b9	1	A30/SG3+	epD6/7
KP3-7	pos	3	DP-50/hv3019b9	1	DPK8/Vd+	epD6/7
KP3-8	pos	3	DP-50/hv3019b9	1	DPK9/O12	epD6/7
KP3-9	pos	3	DP-50/hv3019b9	1	DPK9/O12	
KP3-1O	pos	3	DP-50/hv3019b9	1	DPK9/O12	
KP3-11	pos	3	DP-50/hv3019b9	1	DPK9/O12	
KP3-12	pos	3	DP-46/3d216	1	DPK9/O12	

TABLE 2a. ANALYSIS OF $_{\gamma 1}\kappa$ FAB/PHAGE CLONES

[1]   nomenclature: prefix "KPO" denotes "$_{\gamma 1}\kappa$Fab/phage library, panning 0", "KP1" denotes "$_{\gamma 1}\kappa$Fab/phage library, panning 1", etc.

[2]   agglutination negative or positive against Rh(D)-positive RBC

[3]   Ig heavy chain variable region gene family per Tomlinson *et al.*, *supra*

[4]   closest related Ig heavy chain variable region gene per Tomlinson *et al. supra*

[5]   Ig light chain variable region gene family per Tomlinson *et al.*, *supra*

[6] closest related Ig light chain variable region gene per Tomlinson *et al.*, supra

[7]   Rh(D) epitope as defined by rare RBC agglutination pattern (see Figure 5 and text)

CLONE[1]	AGGLU[2]	VH FAM[3]	VH GENE[4]	Vκ FAM[5]	Vκ GENE[6]	D EPITOPE[7]
LPO-1	neg	4	DP-65/3d75d	1	DPL7/IGLV1S2	
LPO-4	neg	4	DP-70/4d68	6	IGLV8A1	
LPO-3	neg	6	DP-74/VH-VI	3	DPL18/VL7.1	
LPO-4	neg	3	DP-29/12-2	1	DPL3/Iv122	
LPO-5	neg	3	DP-38/9-1	6	IGLV6S1/LV6SW-G	
LPO-6	neg	1	4M28	1	DPL3/Iv122	
LPO-7	neg	1	8M27	1	DPL2/Iv1L1	
LPO-8	neg	5	DP-58/V5-51	6	IGLV6S1/LV6SW-G	
LPO-9	neg	5	DP-73/V5-51	1	DPL7/IGLV1S2	
LPO-10	neg	3	DP-38/9-1	1	DPL2/Iv1L1	
LPO-11	neg	3	DP-31/V3-9P	3	DPL23/VLIII.1	
LPO-12	neg	--	no heavy chain	1	DPL7/IGLV1S2	
LPO-13	neg	3	DP-47/V3-23	--	no light chain	
LPO-14	neg	4	DP-71/3d197d	6	IGLV6S1/LV6SW-G	
LPO-15	neg	4	DP-70/4d68	4	IGLV8A1	
LPO-16	neg	3	DP-54/V3-7	7	DPL19	
LP2-1	pos	3	DP-50/hv3019b9	1	DPL2/Iv1L1	epD2
LP2-2	pos	3	DP-77/WHG16	1	DPL3/Iv122	
LP2-3	pos	3	DP-49/1.9111	1	DPL3/Iv122	epD1
LP2-4	neg	4	3d279d+	1	DPL2/Iv1L1	
LP2-5	pos	3	DP-49/1.9111	3	DPL16/IGLV3S1	epD5
LP2-6	pos	3	DP-50/hv3019b9	1	DPL7/IGLV1S2	epd2
LP2-7	pos	3	b28m	1	DPL7/IGLV1S2	epD2
LP2-8	pos	3	DP-49/1.9111	3	IGLV3S2=Iv318	epD1

TABLE 2b. ANALYSIS OF $_{\gamma 1}\lambda$FAB/PHAGE CLONES

TABLE 2b. ANALYSIS OF $_{\gamma1}\lambda$FAB/PHAGE CLONES						
CLONE[1]	AGGLU[2]	VH FAM[3]	VH GENE[4]	Vκ FAM[5]	Vκ GENE[6]	D EPITOPE[7]
LP2-9	pos	3	DP-50/hv3019b9	3	DPL16/IGLV3S1	epD2
LP2-10	pos	3	DP-77/WHG16	1	DPL3/LV122	
LP2-11	neg	1	DP-75-VI-2	1	DPL5/LV117d	
LP2-12	pos	3	DP-77/WHG16	1	DPL2/LV1L1	epD2
LP2-13	pos	3	COS-8/hv3005f3	4	IGLV8A1	
LP2-14	pos	3	DP-49/1.9111	1	DPL7/IGLV1S2	epD5
LP2-15	pos	3	DP-50/hv3019b9	1	DPL16/IGLV3S1	
LP2-16	pos	3	DP-49/1.9111	2	Iv2046	epd1
LP2-17	pos	3	DP-77/WHG16=V3-21+	1	DPL3/Iv122	epD3/9
LP2-18	pos	3	DP-49/1.9111	2	VL2.1~DPL10/Iv2066	epD1
LP2-19	pos	3	DP-50/hv3019b9	3	DPL16/IGLV3S1	epD2
LP2-20	neg	3	V3-49+	3	DPL16/IGLV3S1	
LP2-21	pos	3	DP-50/hv3019b9	7	DPL18/VL7.1	epD6/7
LP2-22	pos	3	DP-49/1.9111	2	Iv2046	
LP2-23	pos	3	DP-49/1.9111	3	DPL16/IGLV3S1	epD5
LP2-24	pos	3	DP-77/WHG16	1	DPL3/Iv122	
LP2-25	pos	3	DP-50/hv3019b9	7	DPL18/VL7.1	epD6/7
LP2-26	pos	3	DP-49/1.9111	3	DPL16/IGLV3S1	
LP2-27	neg	3	COS-6/DA-8	2	VL2.1	
LP2-28	pos	3	COS-8/hv3005f3	4	IGLV8A1	
LP2-29	pos	3	DP-49/1.9111		DPL13	
LP2-30	pos	3	DP-50/hv3019b9	3	DPL16/IGLV3S1	

TABLE 2b. ANALYSIS OF $_{\gamma1}\lambda$FAB/PHAGE CLONES						
CLONE[1]	AGGLU[2]	VH FAM[3]	VH GENE[4]	Vκ FAM[5]	Vκ GENE[6]	D EPITOPE[7]
LP2-31	pos	3	DP-50/hv3019b9	7	DPL18/VL7.1	
LP2-32	pos	3	DP-49/1.9111	1	DPL2/Iv1L1	
LP2-33	pos	3	DP-50/hv3019b9	7	DPL18/VL7.1	
LP2-34	pos	3	DP-50/hv3019b9	7	DPL18/VL7.1	
LP2-35	pos	3	DP-50/hv3019b9	3	DPL16/IGLV3S1	
LP2-36	pos	3	DP-50/hv3019b9	3	DPL16/IGLV3S1	
LP3-1	pos	3	DP-50/hv3019b9	3	DPL16/IGLV3S1	epD2
LP3-2	pos	3	DP-49/1.9111	3	DPL16/IGLV3S1	epD1
LP3-3	pos	3	DP-49/1.9111	3	DPL16/IGLV3S1	
LP3-4	pos	3	DP-50/hv3019b9	7	DPL18/VL7.1	epD6/7
LP3-5	pos	1	DP-49/1.9111	1	DPL5/LV117d	epD5
LP3-6	pos	3	DP-49/1.9111	1	DPL5/LV117d	epD1
LP3-7	pos	3	DP-77/WHG16	1	DPL2/Iv1L1	epD5
LP3-8	pos	3	b28m	1	DPL7/IGLV1S2	epD2
LP3-9	pos	3	DP-50/hv3019b9	3	DPL16/IGLV3S1	epD2
LP3-1O	pos	3	DP-50/hv3019b9	3	DPL16/IGLV3S1	
LP3-11	pos	3	DP-50/hv3019b9	3	DPL16/IGLV3S1	epD2
LP3-12	pos	3	COS-8/hv3005f3	4	IGLV8A1	epD6/7
LP3-13	pos	3	DP-50/hv3019b9	1	DPL2/Iv1L1	epD2
LP3-14	pos	3	DP-49/1.9111	3	DPL16/IGLV3S1	
LP3-15	pos	3	DP-77/WHG16	1	DPL3/Iv122	epD1
LP3-16	pos	3	DP-49/1.9111	1	DPL2/Iv1L1	epD5
LP3-17	pos	3	DP-50/hv3019b9	3	DPL16/IGLV3S1	

TABLE 2b. ANALYSIS OF $_{\gamma 1}\lambda$ FAB/PHAGE CLONES

CLONE[1]	AGGLU[2]	VH FAM[3]	VH GENE[4]	Vκ FAM[5]	Vκ GENE[6]	D EPITOPE[7]
LP3-18	pos	3	DP-50/hv3019b9	3	DPL16/IGLV3S1	
LP3-19	pos	3	DP-50/hv3019b9	3	DPL16/IGLV3S1	epD5
LP3-20	pos	3	DP-50/hv3019b9	1	DPL2/Iv1L1	
LP3-21	pos	3	DP-49/1.9111	1	DPL3/Iv122	
LP3-22	pos	3	COS-8/hv3005f3	1	DPL2/Iv1L1	
LP3-23	pos	3	DP-49/1.9111	3	DPL16/IGLV3S1	
LP3-24	pos	3	DP-50/hv3019b9	3	DPL16/IGLV3S1	

[1] nomenclature: prefix "LP0" denotes "$_{\gamma 1}\lambda$Fab/phage library, panning 0", "LP1" denotes "$_{\gamma 1}\lambda$Fab/phage library, panning 1", etc.

[2] agglutination negative or positive against Rh(D)-positive RBC

[3] Ig heavy chain variable region gene family per Tomlinson *et al.*, *supra*

[4] closest related Ig heavy chain variable region gene per Tomlinson *et al.*, *supra*

[5] Ig light chain variable region gene family per Tomlinson *et al.*, supra

[6] closest related Ig light chain variable region gene per Tomlinson *et al.*, *supra*

[7] Rh(D) epitope as defined by rare RBC agglutination pattern (see Figure 5 and text)

TABLE 3. SUMMARY OF FAB/PHAGE CLONAL ANALYSIS

Number of unique heavy chains	25
Number of unique κ light chains	18
Number of unique λ light chains	23
Number of $_{\gamma 1}$κ antibodies	20
Number of $_{\gamma 1}$λ antibodies	30
Number Rh(D) epitope specificities represented	5

Use of Fab/Phage Antibodies as Blood Bank Typing Reagents

The ability of the anti-Rh(D) Fab/phage preparations to accurately distinguish Rh(D)-negative from Rh(D)-positive RBCs in microplate hemagglutination assays (Figures 4 and 5) provided evidence that a gel test (Lapierre et al., 1990, Transfusion 30:109-1130) used by blood banks to phenotype RBCs using conventional antisera could be adapted for use with Fab/phage.

The gel test comprises a plastic card of approximately 5 x 7 cm, containing 6 mini-columns each filed with about 20 μl of dextran-acrylamide beads suspended in anti-human globulin (Coombs reagent). Red cells to be typed are incubated with the desired human anti-sera and are centrifuged through the gel. RBCs which are positive for antigens to which the antisera is directed agglutinate as they encounter the anti-human globulin and become trapped in or above the gel matrix. Unreactive RBCs sediment through the gel particles and form a pellet at the bottom of the microtube. Because the gel test offers a number of advantages over traditional blood banking methods for RBC phenotyping including decreased reagent volumes, the elimination of a cell washing step and a more objective interpretation of results, many blood bank facilities have adapted this new technology. As shown in Figure 6, anti-Rh-(D) Fab/phage can be used with gel cards that are modified to contain anti-M13 antibody.

To perform the assay, Rh(D)-negative or -positive red blood cells were incubated with dilutions of anti-Rh(D) Fab/phage ($_{\gamma 1}\kappa$ library, panning #2) and were centrifuged into micro-columns containing beads suspended in anti-M13 antibody. Undiluted Fab/phage stock had a titer of 5×10^{12} cfu/ml similar to that in the microplate settling assay (Figure 4). Because the volume of Fab/phage used in this assay is one-fourth of that in the microplate assay, the amount of Fab/phage present in the 1/625 dilution is approximately equal to that present in the 1/2048 dilution in Figure 4. Therefore, the number of Fab/phage required to yield a positive result is essentially equivalent in both assays.

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In other assays which were performed as just described, when anti-M13 antibody was eliminated from the assay, no agglutination of red blood cells was observed. In addition, anti-IgG antibody does not react with recombinant Fabs expressed on the surface of the bacteriophage. Only Rh-positive cells which were reacted with anti-Rh phage were agglutinated when anti-M13 antibody was present in the assay. It should be noted that when high concentrations of anti-M13 antibody were used, even Rh-negative cells appeared to be agglutinated. This is an artifact resulting from the cross-linking of unbound (*i.e.*, non-reacted) phage which becomes crosslinked in the presence of high amounts of anti-M13 antibody and forms a semi-impenetrable mat through which not all the Rh-negative cells can traverse. In the experiments described herein, an anti-M13 concentration of about 100 µg/ml was considered to be optimal for agglutination and for the prevention of false positive results. Depending on the precise concentrations of reagents and cells used in the assay, the concentration of anti-M13 may deviate from this number.

To assess the relative sensitivity of an anti-M13 modified Micro Typing System, the columns of the Micro Typing System cards had added to them 100 µg/ml of anti-M13 antibody. Rh-negative or Rh-positive red blood cells were incubated with undiluted or with five-fold serial dilutions (1/5, 1/25, 1/125, 1/625 and 1/3125) of anti-Rh phage antibodies. The cards were centrifuged and samples were assessed for agglutination. The modified Micro Typing System card assay was capable of detecting anti-Rh agglutination at a dilution of between 1/625 and 1/3125.

Procedures for isolation of tumor-specific antibodies

Fab/phage specific for tumor cells are useful for *in vitro* diagnosis (lab assays of biopsy, fluid, or blood samples), *in vivo* labeling of tumor/metastasis (coupling of antibody to imaging probe), or for treatment of malignancy (coupling of antibodies to chemical or radioactive toxins). Tumor-specific antibodies are also useful for the identification of novel antigens or markers on tumor cells which may form the basis for anti-tumor vaccines. Further, tumor-specific antibodies useful for the generation of anti-idiotypic antibodies may also form the basis for anti-tumor vaccines.

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Anti-tumor antibodies are generated essentially as described herein for the generation of anti-Rh antibodies. Tumor cells, for example, but not limited to, malignant melanoma cells, are cell-surface biotinylated, labeled with streptavidin-magnetic microbeads, and are then mixed with excess normal melanocytes. Fab/phage libraries are generated from peripheral blood lymphocytes of melanoma patients who possess therapeutically useful anti-tumor antibodies. A number of melanoma patients who have "cured" themselves apparently have done so by mounting a humoral (*i.e.*, antibody) immune response. These Fab/phage libraries are incubated with the admixture of cells. Fab/phage which are directed against epitopes specific for malignant cells will bind to the malignant cells and may then be isolated utilizing the magnetic column panning approach.

Isolation of Fab/phage that identify bacterial virulence factors

The approach described herein may be used to isolate Fab/phage capable of detecting differences between the virulent bacteria and their nonpathogenic counterparts. In this case, the virulent strain of bacteria is magnetically labeled, diluted with the non-pathogenic counterpart, and an Fab/phage library which is generated from lymphocytes obtained from individuals infected with the virulent strain is added. Fab/phage which are isolated in this manner may be useful for the identification of novel bacterial antigens against which antibacterial compounds and/or vaccines may be developed.

Example 2

Genetic and Immunological Properties of

Phage-Displayed Human Anti-Rh(D) Antibodies

Clinically, the human Rh(D) antigen is the most important red blood cell (RBC) membrane protein in transfusion medicine. The alloimmune response against Rh(D) produces high affinity IgG antibodies which cause hemolytic transfusion reactions and hemolytic disease of the newborn (HDN). The prophylactic use of Rh(D)-immune globulin in pregnant Rh(D)-negative women has been a major advance

in the prevention of HDN, yet the mechanism by which the drug exerts its immune modulatory effect is not well understood.

Monoclonal antibodies derived from the B cells of Rh(D)-immune globulin donors have defined several dozen Rh(D) epitopes (Scott, 1996, Transfus. Clin. Biol. 3:333). Paradoxically, the Rh(D) antigen, a *circa* 30 kD transmembrane protein, has minimal extracellular mass and presents a very limited surface area for epitope expression. Because molecular cloning of a large repertoire of anti-Rh(D) antibodies has not previously been performed, these observations remain non-reconciled.

Rational development of recombinant formulations of Rh(D)-immune globulin would be facilitated by molecular cloning of a large number of anti-Rh(D) antibodies. Such cloning would also aid in the design of therapeutic agents that block antibody binding. Furthermore, comprehensive genetic analysis of anti-Rh(D) antibodies within a given alloimmunized individual would serve as a paradigm for human immune repertoire development, an area of which limited information is currently available. Previously, no more than 8 IgG anti-Rh(D) human monoclonal antibodies have been derived from a single individual (Boucher et al., 1997, Blood 89:3277).

In Example 1, a technique useful for isolating Fab/phage antibodies directed against antigens expressed on cell surfaces was described. Using this technique and intact human red blood cells (RBCs), highly diverse $\gamma_1\kappa$ and $\gamma_1\lambda$. Fab/phage libraries against the Rh(D) antigen from the B cells of a single Rh(D)-immune globulin donor were generated.

In this Example, a detailed genetic and serological analysis of 53 unique anti-Rh(D) antibodies derived from 83 randomly chosen clones is presented. These data demonstrate extensive genetic homology between antibodies directed against different Rh(D) epitopes. Evidence is provided herein that antibodies directed against different epitopes can be clonally related. Finally, a model is described which

reconciles the serological diversity of anti-Rh(D) antibodies with the topological constraints imposed by the Rh(D) antigen.

The materials and methods used in the experiments presented in this Example are now described.

Production of Monoclonal Anti-Rh(D) Phage-Displayed and Soluble Fab Molecules

Methods for the isolation of human anti-Rh(D)-specific antibodies from $\gamma_1 \kappa$ and $\gamma_1 \lambda$ Fab/phage display libraries using the pComb3H phagemid vector and a cell-surface panning protocol have been described (Siegel et al., 1997, J. Immunol. Meth. 206:73). Soluble anti-Rh(D) Fab preparations for inhibition studies were produced from bacterial cultures transfected with plasmid DNA from which the M13 gene III coat protein sequence had been excised as described (Siegel et al., 1994, Blood 83:2334; Barbas et al., 1991, Methods: A Companion to Meth. Enzymol. 2:119). Cultures were grown by shaking at 300 RPM at 37°C in superbroth (30 g/L tryptone, 20 g/L yeast, 10 g/L MOPS, pH 7.00) containing 20 mM $MgCl_2$ and 50 mg/ml carbenicillin to an OD_{600} of 0.5. Isopropyl-β-D-thiogalactopyranoside (IPTG) was added to 1 mM and cultures were shaken overnight at 30°C. Bacterial pellets were harvested and resuspended in 1/50th of the initial culture volume with osmotic shock buffer (500 mM sucrose, 1 mM EDTA, 100 mM Tris, pH 8.00), incubated for 30' at 4°C, and centrifuged at 16,000 × g for 15' at 4°C. Fab-containing supernatants were dialyzed against PBS and used in agglutination experiments without further purification.

Anti-Rh(D) Antibody Binding Assays

The binding of anti-Rh(D) Fab/phage or soluble Fab molecules to normal or partial Rh(D) antigens was assessed by indirect agglutination assays as described (Siegel et al., 1994, Blood 83:2334; Siegel et al., 1997, J. Immunol. Meth. 206:73). Briefly, 100-μl aliquots of phage-displayed Fabs or soluble Fabs were incubated with 50 μl of a 3% suspension of RBCs. Following a one-hour incubation at 37°C, the RBCs were washed 3 times with 2 ml of cold PBS to remove unbound antibody. The resulting RBC pellets were resuspended in 100 μl of a 10 μg/ml solution

of sheep anti-M13 antibody (5 Prime-3 Prime, Boulder, CO) for Fab/phage

experiments or goat anti-human κ or λ light chain antibody (Tago, Burlingame, CA)

for $\gamma_1\kappa$ or $\gamma_1\lambda$ soluble Fab experiments, respectively. The RBC suspensions were

transferred to the round-bottomed wells of a 96-well microplate and left undisturbed for

2 hours. Negative reactions show sharp ~2 millimeter diameter RBC spots whereas the

RBCs in agglutinated wells form a thin carpet coating the entire floor of the well

(Siegel et al., 1997, J. Immunol. Meth. 206:73). Agglutination titers for recombinant

antibodies were determined by performing serial 2-fold dilutions in 1% BSA/PBS.

Typically, Fab/phage had agglutination titers of 1/1024 to 1/2048 (where "neat" is

defined as 5×10^{12} cfu/ml; Siegel et al., 1997, J. Immunol. Meth. 206:73) and soluble

Fabs had agglutination titers of 1/64 to 1/128 when prepared as described above.

For determining Rh(D) epitope specificity for anti-Rh(D) Fab/phage

antibodies, the following reference Rh(D) variant cells were used: O/D^{IIIa}Cce, G

positive; B/D^{IIIc}Cce; A/D^{IVa}ce; A/D^{IVa}ce; O/D^{IVa}ce; O/D^{IVb}Cce; B/D^{IVb}Cce, Go^a

negative, Rh32 negative; O/D^{Va}Cce; O/D^{Va}cEe, D^w positive; O/D^{VI}Cce; B/D^{VI}Cce;

AB/D^{VI}Cce; A/D^{VI}cEe; O/D^{VII}Cce; and O/D^{VII}Cce. Each Fab/phage antibody was

tested on at least 3 separate occasions against at least 2 different examples of each

variant cell type and identical epitope assignments were obtained each time. For

antibodies that demonstrated not-previously-described patterns of reactivity or

repeatedly weak reactivity against one type of cell, monoclonal Fab/phage were

prepared on a least 4 separate occasions to verify the patterns of reactivity.

For inhibition studies, the ability of antibodies with different Rh(D)

epitope specificities to compete with each other for binding was assessed by preparing

stocks of each clone in both a soluble Fab form and a phage-displayed form. Pair-wise

combinations of soluble Fabs and Fab/phage were prepared and added to

Rh(D)-positive RBCs. The resulting incubation mixes comprised 50 µl of a 3%

suspension of RBCs, 100 µl of undiluted soluble Fab, and 100 µl of Fab/phage diluted

to its highest agglutinating titer. Following a 1-hour incubation at 37°C, RBCs were

washed, resuspended in anti-M13 antibody, and placed in microplate wells as described

above. That the amount of soluble Fab present in an incubation mixture was sufficient to compete away a Fab/phage that shared the same binding site was determined by verifying that each soluble Fab preparation could block its own Fab/phage.

Inhibition experiments were also performed using pair-wise combinations of soluble Fabs instead of soluble Fab and Fab/phage combinations. In this type of experiment, pairs of soluble Fabs specific for different epitopes were chosen such that one Fab contained a λ light chain and the other a κ light chain. Incubations with RBCs were performed with one Fab in excess and the other in limiting amounts. Blocking of the latter antibody was assessed using a secondary antibody (anti-λ or anti-κ) specific for its light chain isotype.

Nucleotide Sequencing and Analysis

Plasmid DNA for sequencing was prepared using the Qiawell™ system (Qiagen, Chatsworth CA). Double-stranded DNA was sequenced using light chain or heavy chain immunoglobulin constant region reverse primers or a set of unique pComb3H vector primers that anneal 5' to the respective immunoglobulin chain (Barbas et al., 1991, Methods: A Companion to Meth. Enzymol. 2:119; Roben et al., 1995, J. Immunol. 154:6437) and automated fluorescence sequencing (Applied Biosystems, Foster City, CA). Sequence analysis and variable region germline assignments were performed using DNAplot (Althaus et al., 1996, DNAPLOT, http://www.mrc_cpe.cam.ac.uk/imt_doc/DNAsearch.html) and the V Base Directory of Human V Gene Sequences (March 97 update; Tomlinson et al., 1996, V Base Directory of Human V Gene Sequences, http://www.mrc_cpe.cam.ac.uk/imt_doc/ vbase_home_page.html). Germline assignments were corroborated with the MacVector (v. 6.0) software package (Oxford Molecular Group, Oxford, UK) against the same database. Multiple sequence alignments and predictions of isoelectric point were calculated using the Pileup and Isoelectric programs of the GCG software package (v. 8.0.1; GCG, Madison WI). Statistical analysis was performed with Statview (Abacus Concepts, Berkeley CA).

The results of the experiments presented in this Example are now described.

Sequence Analysis of Anti-Rh(D) Heavy and Light Chains

Example 1 describes the use of Fab/phage display and cell-surface panning to isolate a large array of anti-Rh(D) antibodies from the peripheral blood lymphocytes of a single hyperimmunized donor. Separate $\gamma_1\kappa$ and $\gamma_1\lambda$ Fab/phage display libraries were constructed and contained 7×10^7 and 3×10^8 independent transformants, respectively, based on electroporation efficiency. Each library was panned independently using a simultaneous positive/negative selection strategy with magnetically-labeled Rh(D)-positive RBCs and unmodified Rh(D)-negative RBCs as described. Following two rounds of panning, 32 of 36 $\gamma_1\lambda$ and 15 of 15 $\gamma_1\kappa$ clones were positive for anti-Rh(D) activity. After the third round of panning, 24 out of 24 $\gamma_1\lambda$ and 12 out of 12 $\gamma_1\kappa$ clones were positive. Nucleotide sequencing of the 83 positive clones revealed a total of 28 unique heavy and 41 unique light chains. Due to combinatorial effects during phage display library construction, heavy and light chain gene segments paired to produce 53 unique Fab antibodies.

Anti-Rh(D) heavy chains

All of the heavy chain sequences used $V_H III$ family-encoded gene products, as indicated in Figures 7 and 8. Several heavy chain sequences shared identical VDJ joining regions, and 12 unique VDJ rearrangements were identified. These rearrangements were designated VDJ1 through VDJ12. Alignment of these sequences against the V Base Directory of Human V Gene Sequences revealed that only four $V_H III$ genes were used by these antibodies: VH3-21, VH 3-30, VH 3-33, and VH 3-30.3. VH3-21 was used by 1 of the 12 VDJs and 2 of the 28 clones; VH3-30 was used by 1 VDJ and 6 clones; VH3-33 was used by 9 VDJs and 19 clones; and VH3-30.3 was used by 1 VDJ and 1 clone. Interestingly, VH3-30, VH3-33, and VH3-30.3 comprise a set of closely related genes (>98% homology; Figure 8B) and their next nearest neighbor, VH3-07, is only 90% homologous (Figure 8C). Hereafter, these three genes are referred to as the "VH3-33 superspecies". Heavy chain E1

differed from VH3-21 by six mutations and differed from VH3-48 by ten mutations; hence, it was assigned to the former germline gene. Because there were no common mutations among the VH3-33 clones, it is highly probable that the donor possessed the VH3-33 germline gene. However, we could not formally rule out gene duplication with allelic variants of VH3-33 or the existence of variant alleles of the other germline genes in the donor. The isolation of clones sharing multiple VDJ joining regions strongly suggests that cloning artifacts cannot account for the V_H restrictions observed.

Neither J_H nor D segments showed restriction. At least 9 different D segments were used and J_H gene utilization comprised J_H6 (5 VDJs and 9 clones), J_H4 (4 VDJs and 10 clones), J_H3 (2 VDJs and 8 clones) and J_H5 (1 VDJ and 1 clone). All four V_H genes were Chothia class 1-3 (Chothia et al., 1992, J. Mol. Biol. 227:799), and the CDR3s showed a narrow range of length from 15 to 19 residues.

Because rearranged heavy chain genes demonstrate extensive diversity, clones sharing identical VDJ rearrangements are generally considered to have arisen from the same clone. Based upon nucleotide alignment with the germline genes, the ontogeny tree in Figure 9 was constructed for the 12 VDJs and 28 clones. By using the most parsimonious mutation scheme (i.e. postulating the minimum number of mutations), putative intermediate antibodies were derived for several of the VDJs and were designated Ca, Cb, Da, Db, and Dc (Figures 8A and 9). Compared with the isolated heavy chain clones, which had between 6 and 23 nucleotide differences from their germline counterparts, these putative intermediates had between 3 and 12 mutations from germline. Based upon the ontogeny tree, the number of independent mutations could be tabulated among the clones. The most commonly mutated residues were 52a and 58 (7 independent mutations), followed by residues 30, 31 and 50 (6 mutations), and residue 55 (5 mutations). In the VH3-33 superspecies, residues 52a and 58 in CDR2 are tyrosine residues and residue 52a was mutated to phenylalanine in 6 of the 11 VDJs derived from VH3-33 superspecies V_H genes. Mutations at residue 58 comprised glutamate (3), aspartate (2), histidine (1) and asparagine (1). The AGY serines at residues 30, 31 and 55 were mutated to a number of different amino acids,

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although the AGY serine at 82b was conserved in all clones. The valine at residue 50 in the VH3-33 superspecies also had a diverse set of mutations. This distribution of "hot spots" is similar to that seen with non-productive rearrangements as previously reported by Dörner et al (1997, J. Immunol. 158:2779).

Anti-Rh(D) light chains

Seventeen of the 18 κ light chains were from the V_κI family and the remaining light chain originated from a V_κII family member germline gene (Fig 10). Only four V_κ germline genes were used (15 clones were derived from DPK9 alone), and the κ light chain clones had between 1 and 49 mutations from their corresponding V_κ germline genes. All five of the known J_κ genes were used and were each joined to the DPK9 gene in one or more clones. Because the light chains showed considerably less diversity in their joining regions than the heavy chains, it was difficult to assign common clonal origins. However, an ontogeny tree was constructed by grouping common V and J gene segments along with common mutations. Based upon this analysis, the 18 κ chains comprised at least 10 different recombination events.

λ light chains were restricted by their J_λ gene usage but showed no restriction in their use of V_λ genes (Figure 11). The 23 λ light chains all used the J_λ2Vasicek gene but were derived from V_λI (12 clones), V_λIII (5), V_λVII (3), V_λII (2) and V_λIV (1) family genes. The number of mutations ranged from 2 to 41 from the nearest germline V_λ gene. Based upon common joining regions and mutations, these 23 l light chains were derived from at least 13 different B cells.

Assessment of the Diversity of the Non-panned Libraries

In order to determine whether the apparent restriction in gene usage of the anti-Rh(D) antibodies could have been due to pre-selection factors (i.e. cloning artifacts), the diversity of the non-panned γ_1κ and γ_1λ Fab/phage libraries was assessed. By sequencing 39 randomly-picked clones, we determined that there were no duplicate heavy or light chain sequences, and that there was significant heterogeneity in V gene family representation before selection (Figure 12). In fact, the variable region

gene family distribution was not unlike that found by other investigators for IgG-secreting lymphocytes in adult peripheral blood (Stollar, 1995, Ann. NY Acad. Sci. 764:547). Furthermore, of the 14 V_HIII-encoded negative clones, only one used a VH3-33 superspecies germline gene (VH3-30.3); the other 13 were encoded by VH3-07 (3), 3-09 (2), 3-15 (2), 3-48 (2), 3-72 (2), 3-23 (1),and DP-58 (1). Therefore, the restriction of the 83 anti-Rh(D) clones to the VH3-33, 3-30, 3-30.3 and 3-21 genes is significant and not a result of skewed representation of certain germline genes within the originally constructed $\gamma_1\kappa$ and $\gamma_1\lambda$ Fab/phage libraries.

Heavy and Light Chain Contribution to Rh(D) Epitope Specificity

Because of the conformational dependency of Rh(D) antigenicity, Rh(D) "epitopes" have been classically defined through the use of RBCs obtained from rare individuals whose cells appear to produce Rh(D) antigens "lacking" certain epitopes. Examining the pattern of agglutination of a particular anti-Rh(D) monoclonal antibody with such sets of partial Rh(D) RBCs enables one to categorize that antibody's fine specificity.

Monoclonal Fab/phage preparations were prepared in triplicate for each of the 53 anti-Rh(D) clones and tested against a panel of Rh(D) category cells IIIa/c, IVa, IVb, Va, VI, and VII. This panel of cells can differentiate between the Rh(D) epitope specificities as described by Lomas et al. (1989, Vox Sang 57:261; designated epitopes epD1, epD2, epD3, epD4, epD5, and epD6/7). Agglutination experiments using the Fab/phage clones demonstrated five different patterns of reactivity, including a new pattern which had not been described in the original study by Lomas et al. or in the more recently-described (Scott, 1996, Transfus. Clin. Biol. 3:333; Stollar, 1995, Ann. NY Acad. Sci. 764:547) 9-, 30-, or 37-epitope systems (as indicated by the data depicted in Figures 13 and 14). Although nearly all Fab/phage gave unequivocal agglutination reactions, a few antibodies gave repeatedly weak patterns of reactivity against one of the panel cells. For these reactions, monoclonal Fab/phage were prepared on at least 4 separate occasions to verify the patterns of reactivity.

The most commonly-recognized epitope was epD6/7, against which 13 of the clones described herein were directed. Interestingly, monoclonal anti-Rh(D) clones isolated using conventional tissue culture methods are most often specific for epD6/7 (Mollison et al., 1993, In: Blood Transfusions in Clinical Medicine, 9th ed., Blackwell Scientific, Oxford, U.K.). EpD2, epD1, and epD3 were recognized by 10, 7, and 2 clones, respectively. Six clones agglutinated cells of categories IIIa/c, IVa, and VII, but not of categories IVb, Va, and VI, and were designated anti-"epDX". This pattern is identical to epD1, except that the IVa cell is agglutinated. Three clones gave intermediate reactions with cell IVa, but otherwise showed patterns consistent with epDX or epD1. These clones were designated epDX^1 or epD1^X depending on whether this reactivity against cell IVa was stronger or weaker, respectively (see Figure 14). Similarly, reaction patterns for epD1 and epD2 differ by a positive reaction with the category Va cell; therefore, one clone was given epD2^1 specificity because it gave only moderate reactivity against that cell. Such variable reactions against one or more partial Rh(D) cells have been observed for anti-Rh(D) monoclonal antibodies produced through conventional tissue culture methods (Tippett et al., 1996, Vox Sang. 70:123).

Because of the reassortment of heavy and light chain gene segments that occurs during the construction of a phage display library, a number of clones were isolated that shared either a heavy (e.g. E1) or light (e.g. M3) chain sequence (Fig 14). Some heavy chains were found to have paired with both κ and λ light chains (e.g. C1, D20) and each demonstrated anti-Rh(D) specificity. Interestingly, some heavy chains (e.g. E1, D12) mapped to different epitopes depending upon the light chains with which they were paired. In particular, the light chains of two such clones, E1/M2 and E1/M3, differed by only three amino acid residues (Fig 11) and these differences appear to confer specificity for epD2 vs. epD3.

Inhibition Studies

To investigate the topological relationships among the Rh(D) epitopes, inhibition studies were performed. Gorick et al. (1988, Vox Sang. 55:165) used pairs of non-labeled and ^{125}I-labeled anti-Rh(D) monoclonal antibodies to demonstrate that

antibodies to at least three different Rh(D) epitopes (subsequently identified as epD1, D6 and D7; Lomas et al., 1989, Vox Sang. 57:261) could inhibit one another. Recombinant antibodies to five Rh(D) epitopes were used to confirm and extend those findings (Figure 15). In one series of experiments, the ability to express each antibody in both a soluble Fab as well as phage-displayed form was exploited to determine whether a soluble Fab directed against one epitope would inhibit the agglutination induced by an Fab/phage directed against a different epitope. Reciprocal pairs of soluble Fab and Fab/phage specific for epD1, epD2, epD3, epD6/7, and epDX were tested. All ten combinations showed mutual inhibition patterns (illustrated in Figure 15A for an anti-epD3/anti-epD6/7 combination). To show that this inhibition was not due to non-specific factors, a control with an irrelevant RBC-binding recombinant antibody (an anti-blood group B antibody) was performed (Figure 15B). That sufficient inhibitory amounts of soluble Fab was present were first verified by demonstrating that each soluble Fab could inhibit its own Fab/phage (Figures 15A and 15B; samples on diagonal). Similar results were obtained using pairs of soluble Fabs which differed in their light chain isotype composition (Figure 15C).

Isoelectric Point Analysis of Anti-Rh(D) Antibodies

The restriction in V_H germline gene usage to only four V_HIII family members was intriguing in light of their ability to confer specificity to a number of Rh(D) epitopes. V_H germline gene segments used to encode anti-Rh(D) antibodies are among the most cationic segments available in the human V_H repertoire which may be used to account for the relatively high pI of polyclonal anti-Rh(D)-containing antisera (Boucher et al., 1997, Blood 89:3277; Abelson et al., 1959, J. Immunol. 83:49; Frame et al., 1969, Immunology 16:277). Although the cationic nature of the antibodies may be important for binding to Rh(D), a constitutive net positive charge may be necessary to permeate the highly negative RBC ζ potential, thus permitting antibody to contact antigen (Mollison et al., 1993, In: Blood Transfusion in Clinical Medicine, 9th ed., Blackwell Scientific, Oxford, U.K.). In either case, analysis of the predicted pI for the 28 heavy chains and 41 light chains isolated here showed an interesting phenomenon

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for the heavy chains, as compared with the light chains. Using the pI interval scale of Boucher et al. (1997, Blood 89:3277), the average pI of the four germline V_H segments used to encode the 28 heavy chains is high (9.87 ± 0.15), significantly higher than that of 39 randomly-picked, non-Rh(D) binding clones from the original non-panned libraries (9.24 ± 0.80, P < 10^{-5}). Similar to the results of Boucher et al., the addition of D and J_H segments and the introduction of somatic mutation did not significantly change the pI of the average anti-Rh(D) heavy chain (9.81 ± 0.33, P < 0.37). However, for the light chains, the average pI of their germline counterparts was not cationic, but the light chains became so through the addition of J_L segments and somatic mutation. Overall, for all 18 κ and 23 λ light chains, paired t-test analyses before and after somatic mutation showed a significant increase in net positive charge when comparing germline V_L (6.63 ± 1.47) with expressed V_L (7.28 ± 1.51, P < 10^{-3}) or germline $V_L J_L$ (7.43 ± 1.47) with expressed $V_L J_L$ (8.55 ± 1.35, P < 10^{-7}). There was no significant increase in a similar analysis of 16 non-Rh(D) binding clones (P < 0.59 and P < 0.19, respectively). Examination of the light chain sequences listed in Figures 10 and 11 revealed that this increase in pI resulted from mutations that not only introduced positively-charged residues, but also eliminated some negatively-charged residues. There were 31 such events, 29 (91%) of which occurred in the light chain CDR regions.

Conventional and Phage-Displayed Anti-Rh(D) Monoclonal Antibodies

The phage-display derived anti-Rh(D) clones were compared with those produced by conventional tissue culture techniques (EBV-transformation and cell fusion). Despite the relatively small number of previously-published sequences for IgG anti-Rh(D) antibodies (N=21) and the fact that they were derived from over 10 different donors, there was surprisingly good correlation between the two groups, as indicated in Table 3. Both cohorts demonstrated a predominance of V_HIII-family encoded germline genes, particularly those of the VH3-33 superspecies. CDR3 regions had similar lengths ranging from 15-19 residues for Fab/phage antibodies and 16-20 for conventional monoclonal antibodies, although one heterohybridoma was an outlier,

having a CDR3 length of 28 residues. κ light chains were biased towards $V_\kappa 1$ family members and λ light chains demonstrated the preferential use of the $J_\lambda 2$Vasicek gene. The only qualitative discrepancy was in V_λ family usage where Fab/phage clones demonstrated a slight preference for $V_\lambda I$ vs. $V_\lambda III$ family members for conventional monoclonal antibodies. However, in both cohorts, DPL16 was used more often than any other λ light chain gene.

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Table 3. Comparison of IgG Fab/phage library-derived anti-Rh(D) monoclonal antibodies prepared as described herein with those previously produced by conventional tissue culture methods

Attribute	Previously Published*	(by clone)†	Current Study	(by VDJ)
Heavy Chains				
VH3 family derived	12/21 (57%)	28/28 (100%)		12/12 (100%)
VH3-33 superspecies‡/VH3	10/12 (83%)	26/28 (93%)		11/12 (92%)
VH3-33/VH3	9/12 (75%)	19/28 (68%)		9/12 (75%)
VH3-21/VH3	1/12 (8%)	2/28 (7%)		1/12 (8%)
VH4-34 derived	2/21 (10%)	0/28 (0%)		0/12 (0%)
JH6 usage	15/21 (71%)	9/28 (32%)		5/12 (42%)
CDR3 length	16-20 (28§)		15-19	
κ Light Chains				
Vκ1 family derived/total κ	8/12 (67%)		17/18 (94%)	
Jκ1 usage/total κ	4/12 (33%)		6/18 (33%)	
Jκ2 usage/total κ	4/12 (33%)		6/18 (33%)	
λ Light Chains				
Vλ1 family derived/total λ	2/8 (25%)		12/23 (52%)	
Vλ3 family derived/total λ	5/8 (63%)		5/23 (22%)	
DPL16 derived/Vl3 family	3/5 (60%)		4/5 (80%)	
Jλ2Vasicek usage/total λ	6/8 (75%)		23/23 (100%)	

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Notes for Table 3

*Compiled from a total of 21 sequences of IgG anti-Rh(D) antibodies isolated from multiple subjects originally published by Bye et., Hughes -Jones et al., Chouchane et al., and Boucher et al. and available from Genbank. One light chain (Oak-3) was not available in Genbank and was not included in the assessment.

†For heavy chains, left column tabulates each clone separately; right column tabulates clones on the basis of shared V-D-J joining regions

‡VH3-33 superspecies defined as the group of VH3 family germline genes comprising VH3-33, VH3-30, and VH30.3.

§CDR3 length outlier

It has been suggested in the literature that the VH4-34 (VH4.21) germline gene, a gene used by many autoantibodies and cold agglutinins, may play an important role in the immune response to Rh(D) (Silberstein et al., 1991, Blood 78:2377; Pascuel et al., 1991, J. Immunol. 146:4385; Silverman et al., 1988, J. Exp. Med. 168:2361; Thompson et al., 1991, Scand. J. Immunol. 34:509). However, these conclusions arose from the analysis of *IgM* monoclonal antibodies and only 2 of the 21 published anti-Rh(D) IgG sequences used VH4-34 (Bye et al., 1992, J. Clin. Invest. 90:2481). In a related series of experiments, aliquots of the $\gamma_1\kappa$ and $\gamma_1\lambda$ libraries obtained after the second and third rounds of selection were pooled and then panned against the VH4-34 specific rat anti-idiotypic monoclonal antibody (9G4; Stevenson et al., 1989, Br. J. Haematol. 72:9). Although VH4-34 encoded antibodies were successfully enriched, the Fab/phage were not specific for Rh(D) and displayed serological characteristics similar to those of cold agglutinins.

Rh(D) Epitopes and Significance of Antibody Sequences

Since the initial report by Argall et al. in 1953 (J. Lab. Clin. Med. 41:895), it has been recognized that rare individuals who type as Rh(D)-positive can produce allo-anti-Rh(D) antibodies in response to Rh(D) immunization by transfusion or pregnancy. This phenomenon was explained by hypothesizing that the Rh(D) antigen is a "mosaic structure" and that these individuals were producing alloantibodies

to parts of the mosaic they lack. By systematically examining patterns of reactivity between their cells and sera, RBCs expressing partial Rh(D) antigens were divided into categories, each presumed to have a different abnormality in their Rh(D) antigen. Through the subsequent use of index panels of monoclonal anti-Rh(D) antibodies, a series of epitopes were defined of which the number and combination varied from one Rh(D) category to another. As new monoclonal antibodies were produced, their reactivity profiles against these partial Rh(D) RBCs became the standard method for determining Rh(D) antibody epitope specificity. Molecular analyses of partial Rh(D) phenotypes have shown that the Rh(D) genes in these individuals have either undergone intergenic recombination with the highly homologous Rh(CE) gene, or, less commonly, have sustained point mutation(s) (Cartron et al., 1996, Transfus. Clin. Biol. 3:497).

As noted earlier, to investigate the topological relationships among Rh(D) epitopes, Gorick et al. performed competition experiments with Rh(D) monoclonal antibodies and observed varying degrees of inhibition (Gorick et al., 1988, Vox Sang. 55:165). These results, when combined with those of Lomas et al. (1989, Vox Sang. 57:261), suggested a model for Rh(D) in which epitopes are spatially distinct yet demonstrate a certain degree of overlap as illustrated in Figure 16A. This model explained how antibodies to two different Rh(D) epitopes (in this case epD2 and epD3) could inhibit each other's binding to wild type Rh(D), and how a change in the structure of Rh(D) in category VI RBCs (asterisk in Figure 16A) would cause the loss of epD2. However, based upon this concept of Rh(D) epitopes as distinct domains, one would expect that antibodies against different epitopes of Rh(D) would be structurally and genetically distinct as well. Thus, it was surprising that the anti-Rh(D) clones described herein demonstrated such marked restriction in gene usage. For example, only two superspecies of V_H genes were used despite specificities for 4 of the original 6 Rh(D) epitopes described by Lomas et al. (1989, Vox Sang. 57:261). Furthermore, multiple specificities could arise from a single heavy chain depending upon the light chain with which it was paired (e.g. E1 with M2, M3, L3, or L4). In addition, other

clones repeatedly demonstrated variable weak reactivity against certain Rh(D) category RBCs that would affect the epitope specificities to which they were assigned (e.g. C1 with O1, M1, or J5).

Several hypotheses could account for these findings. The most simplistic interpretation is that the heavy chain does not directly interact with the antigen, but rather is responsible for bringing the antibody in close proximity with the antigen. The specific interactions between the light chain and the antigen would then determine the epitope specificity for that antibody. In this regard, the data presented herein are consistent with the observations of Boucher et al. (1997, Blood 89:3277) on the relative cationic nature of anti-Rh(D) heavy chains. However, because it was determined during the studies described herein that light chains become cationic during somatic mutation, the charge of the entire antibody may play a role in its ability to bind, resulting in the selection and expansion of particular B-cell clones.

A more compelling hypothesis is that Rh(D) epitopes do not differ spatially but differ only in the number and arrangement of contact residues presented, as illustrated in Figure 16B. In other words, the "footprints" of most, if not all, anti-Rh(D) antibodies are essentially identical to one another. The genetic events which produce partial Rh(D) molecules result in the loss of certain critical key points of contact necessary for some antibodies to bind; alternatively, they result in the formation of new structures that interfere with the binding of other anti-Rh(D) immunoglobulins. For example, the introduction of a "ledge" in Rh(D) category VI cells (asterisk in Figure 16B) does not interfere with the binding of an anti-epD3 antibody, but does prevent the binding of anti-epD2. Therefore, category VI RBCs are said to have epD3 but "lack" epD2.

This model is consistent with the inhibition experiments described herein (e.g. Figure 15) and with those of Gorick et al. (1988, Vox Sang. 55:165) and offers an explanation for the marked restriction in heavy chain gene usage. This model also reconciles a mechanism by which one heavy chain (e.g. E1) can confer binding to multiple epitopes and why some of the recombinant anti-Rh(D) antibodies described

herein, as well as some conventionally-produced monoclonal antibodies (e.g. Tippett et al., 1996, Vox Sang. 70:123), display variable reactivity against certain categories of partial Rh(D) RBCs. From the antigen's perspective, this model explains how a single point mutation in Rh(D) can result in the loss of multiple Rh(D) epitopes (such as T283I in category HMi RBCs) and how the residues associated with the expression of some epitopes appear to be distributed among nearly all the extracellular loops of Rh(D). It also provides an understanding as to how ≥37 "epitopes" can fit on the relatively small extracellularly-exposed surface of the Rh(D) molecule.

This concept of "coincident" epitopes is best exemplified by comparing the E1/M2 and E1/M3 clones described herein. The only difference between the reactivity of E1/M2 and E1/M3 is the ability of the latter antibody to agglutinate Rh(D) category VI cells, as depicted in Figure 13. Hence, E1/M2 is classified as an anti-epD2 and E1/M3 as an anti-epD3 antibody. Light chains M2 and M3 differ by only 3 residues: D82A, G95aA, and W96V, as indicated in Figure 11. Therefore, some combination of these three residues confers reactivity against category VI cells. In other words, epD2 and epD3, as seen by the E1/M2 and E1/M3 antibodies, differ by the binding constraints imposed by at most three mutations. If the model depicted in Figure 16A were correct and the epitopes were independent, these mutations would have to cause enough structural alteration in the antibody combining site so that a completely separate epitope on the same antigen would be recognized. It would seem unlikely that these 3 mutations could cause such a change, especially given the lack of internal homology domains in Rh(D). Therefore, it is concluded that it is far more plausible that the footprints of these 2 antibodies are essentially identical, and that one or more of these mutations (e.g. the tryptophan in CDR3 of M2) prevent(s) the interaction of E1/M2 with category VI RBCs. Since other clones demonstrate that the light chain can confer specificity against epD1, epD2, or epD3 (with the E1 heavy chain); epD1 or epDX (with C5); and epD1, epD2, and epD6/7 (with D12), we suggest that all 5 of these epitopes have similar antibody combining sites.

Immunologic and Clinical Implications of Proposed Model

The model depicted in Figure 16B leads to additional predictions concerning the Rh(D) immune response beyond simply clarifying what is meant by an Rh(D) epitope. It is commonly stated in the transfusion medicine literature that individuals whose RBCs express partial Rh(D) antigens are free to make antibodies to the Rh(D) epitopes they lack (Mollison et al., 1993, In: Blood Transfusion in Clinical Medicine, 9th ed. Blackwell Scientific, Oxford, U.K.). Therefore, an individual who produces category VI RBCs should be able to make anti-epD2 but not anti-epD3. If these epitopes were truly independent, then the immune repertoire of the anti-epD2 antibodies made by a category VI individual would be similar to those produced by an Rh(D)-negative person. However, to the immune system, epD2 and epD3 are not independent.

It is herein postulated that somatic mutation of an anti-epD3 antibody can change its fine specificity to that of epD2 (or vice-versa, see Figure 16C). Suppose that the preferred way of making an anti-epD2 antibody is through an anti-epD3 intermediate. To an Rh(D)-negative individual, this process can take place unimpeded. However, in a category VI individual, this route would be unfavorable because an anti-epD3 antibody would be self-reactive. As a result, such an individual would have to make anti-epD2 antibodies by alternative routes or by tolerating some degree of auto-reactivity in the process. With respect to the latter point, it is of interest to note that a transient production of *auto*-anti-Rh(D) frequently precedes or accompanies the early production of *allo*-anti-Rh(D) in individuals who express partial Rh(D) antigens (Chown et al., 1963, Vox Sang. 8:420; Macpherson et al., 1966, J. Clin. Pathol. 45:748; Beard et al., 1971, Med. Genet. 8:317; Cook 1971, Br. J. Haematol. 20:369; Holland et al., Transfusion 13:363 (Abstract); Issit, 1985, In: Applied Blood Group Serology, 3rd ed., Montgomery Scientific, Miami FL). It is predicted, therefore, that the anti-epD2 antibodies from a category VI individual would be different in composition (i.e. gene usage) and quite possibly quantitatively depressed as compared to an Rh(D)-negative individual. This may be analogous to the antibodies of the ABO blood group system in

which it has been observed that anti-A and anti-B titers in blood group O individuals are significantly higher than in blood group B or A individuals, respectively (Ichikawa, 1959, Jap. J. Med. Sci. Biol. 12:1). Blood group O individuals are unconstrained in creating their anti-A and anti-B immune repertoires while individuals who produce A or B antigens (2 nearly identical structures) must do so in a manner that avoids self-reactivity.

In the case of antibodies E1/M2 and E1/M3, they appear to have arisen from a common precursor B cell rather than directly from each other (Figure 11). To test the framework of the hypothesis presented herein, i.e. somatic mutation resulting in "epitope migration" of an antibody, one may construct the precursors and potential intermediates between the M2 and M3 light chains and then determine what Rh(D) epitope specificities (if any) they express. This concept of epitope migration has been previously reported for murine anti-cryptococcal and anti-type II collagen antibodies (Mukherjee et al., 1995, J. Exp. Med. 181:405; Mo et al., 1996, J. Immunol. 157:2440).

If the model proposed herein for Rh(D) epitopes is correct, then the question of the number of epitopes may be obsolete. There may be as many epitopes as can be differentiated by the number of cell categories, i.e. 2^n epitopes where n is the number of distinct partial Rh(D) RBCs.

A more important question is the interrelationships between the various epitopes. For example, are some epitopes "further away" than others -- not in the topological sense, but in terms of the number of mutational hits an antibody needs to receive in order to change its serologic reactivity. Furthermore, does the humoral immune response in a partial Rh(D) individual differ from that in an Rh(D)-negative individual in the manner predicted by this model? One may find that allo-anti-Rh(D) antibodies made by partial Rh(D) individuals are not as clinically significant, i.e. capable of inducing hemolysis. This may explain why hemolytic disease of the newborn due to anti-Rh(D) produced by pregnant individuals with partial Rh(D) phenotypes is so rare even when taking into account the low prevalence of the partial Rh(D) phenotypes (Mollison et al., 1993, In: Blood Transfusion in Clinical Medicine,

9th ed. Blackwell Scientific, Oxford, U.K.). A better understanding of the immune response to Rh(D) in these patients may alleviate concerns regarding the need to identify such individuals to ensure that they only receive Rh(D)-negative blood products for transfusion and Rh(D)-immune globulin during pregnancy (Jones et al., 1995, Trans. Med. 5:171). Furthermore, with respect to the design of recombinant Rh(D)-immune globulin for use in Rh(D)-negative patients, it may not be necessary to formulate cocktails of monoclonal antibodies containing multiple Rh(D) epitope specificities.

Sequence Data

Genbank accession numbers for anti-Rh(D) heavy chains are as follows: B01, AF044419; C01, AF044420; C03, AF044421; C04, AF044422; C05, AF044423; C08, AF044424; C10, AF044425; D01, AF044426; D03, AF044427; D04, AF044428; D05, AF044429; D07, AF044430; D08, AF044431; D09, AF044432; D10, AF044433; D11, AF044434; D12, AF044435; D13, AF044436; D14, AF044437; D15, AF044438; D16, AF044439; D17, AF044440; D18, AF044441; D20, AF044442; D30, AF044443; D31, AF044444; E01, AF044445; E03, AF044446.

Genbank accession numbers for anti-Rh(D) κ light chains are as follows: F01, AF044447; G01, AF044448; H01, AF044449; I01, AF044450; I02, AF044451; I03, AF044452; I04, AF044453; I05, AF044454; I06, AF044455; I07, AF044456; I08, AF044457; I09, AF044458; I10, AF044459; I11, AF044460; I12, AF044461; I13, AF044462; I15, AF044463; I16, AF044464.

Genbank accession numbers for anti-Rh(D) λ light chains are as follows: J01, AF044465; J02, AF044466; J04, AF044467; J05, AF044468; K01, AF044469; K02, AF044470; K03, AF044471; L01, AF044472; L03, AF044473; L04, AF044474; L05, AF044475; M01, AF044476; M02, AF044477; M03, AF044478; N01, AF044479; N02, AF044480; O01, AF044481; O02, AF044482; O03, AF044483; P01, AF044484; Q01, AF044485; R01, AF044486; S01, AF044487.

<u>Amino Acid Sequences of Anti-Rh(D) Heavy and Light Chains</u>

The amino acid sequences of various anti-Rh(D) chains are represented using single letter amino acid codes, as described herein.

The amino acid sequence of the anti-Rh(D) chain B01 is

EVQLLESGGGVVQPGRSLRLSCAASGFTFRSYAMHWVRQAPGKGLEWVAAT AYDGKNKYYADSVKGRFTISRDNSKNTLFLQMNSLRAEDTAVFYCARGGFYY DSSGYYGLRHYFDSWGQGTLVTVSS (SEQ ID NO: 1).

The amino acid sequence of the anti-Rh(D) chain C01 is

EVQLLESGGGVVQPGRSLRLSCAASGFSFSSYGMHWVRQAPGKGLEWVSVIS YDGHHKNYADSVKGRFTISRDNSKKTLYLQMNSLRPEDTAVYYCANLRGEVT RRASVPFDIWGPGTMVTVSS (SEQ ID NO: 2).

The amino acid sequence of the anti-Rh(D) chain C03 is

EVQLLESGGGVVQHGRSLRLSCAASGFSFSSYGMHWVRQAPGKGLEWVSVIS YDGHHKNYADSVKGRFTISRDNSKKTLYLQMNSLRPEDTAVYYCANLRGEVT RRASVPFDIWGPGTMVTVSS (SEQ ID NO: 3).

The amino acid sequence of the anti-Rh(D) chain C04 is

EVQLLESGGGVVQPGRSLRLSCAASGFSFSTYGMHWVRQAPGKGLEWVSVIS YDGHNKNYADSVKGRFTISRDNSKKTLYLQMNSLRPEDTAVYYCANLRGEVT RRASIPFDIWGQGTMVTVSS (SEQ ID NO: 4).

The amino acid sequence of the anti-Rh(D) chain C05 is

EVQLLESGGGVVQPGRSLRLSCAASGFSFSSYGMHWVRQAPGKGLEWVAVIS YDGTNKYFADSVKGRFTISRDNSKKTLYLQMTSLRPEDTAVYFCANLRGEVTR RASVPLDIWGQGTMVTVSS (SEQ ID NO: 5).

The amino acid sequence of the anti-Rh(D) chain C08 is

EVQLLESGGGVVQPGRSLRLSCAASGFSFSSYGMHWVRQAPGKGLEWVAVIS YDGTNKYFADSVKGRFTISRDNSKKTLYLQMTSLRPEDTAVYFCANLRGEVTR RASVPLDIWGQGTMVTVSS (SEQ ID NO: 6).

The amino acid sequence of the anti-Rh(D) chain C10 is

EVQLLESGGGVVQPGRSLRLSCAASGFSFSSYGMHWVRQAPGKGLEWVSVIS

YDGHHKNYADSVKGRFTISRDNSKKTLYLQMNSLRPEDTAVYYCANLRGEVT

RRASVPFDIWGPGTLVTVSS (SEQ ID NO: 7).

The amino acid sequence of the anti-Rh(D) chain D01 is

EVQLLESGGGVVQPGRSLRLSCVVSGFTFNNYGMHWVRQAPGKGLEWVAVI

WFDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARENQIK

LWSRYLYYFDYWGQGTLVTVSS (SEQ ID NO: 8).

The amino acid sequence of the anti-Rh(D) chain D03 is

EVQLLESGGGVVQPGRSLRLSCAASGFTFSTYGMHWVRQAPGKGLEWVAVI

WFDGSNKEYADSVKGRFTVSRDNSKNTLYLQMNSLRAEDTAVYYCAREEVV

RGVILWSRKFDYWGQGTLVTVSS (SEQ ID NO: 9).

The amino acid sequence of the anti-Rh(D) chain D04 is

EVQLLESGGGVAQPGRSLRLSCVASGFSLRSYGMHWVRQAPGKGLEWVADI

WFDGSNKDYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDWRV

RAFSSGWLSAFDIWGQGTMVTVSS (SEQ ID NO: 10).

The amino acid sequence of the anti-Rh(D) chain D05 is

EVQLLEESGGGVAQPGRSLRLSCVASGFSLRSYGMHWVRQAPGKGLEWVADI

WFDGSNKDYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDWRV

RAFSSGWLSAFDIWGQGTTVSVSS (SEQ ID NO: 11).

The amino acid sequence of the anti-Rh(D) chain D07 is

EVQLLESGGGVVQPGRSLRLSCAVSGFTLTNYGMHWVRQAPGKGLEWVAHV

WYDGSKTEYADSVKGRFAVSRDKSKNTLFLQMNSLTAEDTAIYYCARERREK

VYILFYSWLDRWGQGTLVTVSS (SEQ ID NO: 12).

The amino acid sequence of the anti-Rh(D) chain D08 is

EVQLLEESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGRGLEWVALI

WYDGGNKEYADSVKGRFSISRDNSKNTLYLQVNSLRADDTAVYYCARDQRA

AAGIFYYSRMDVWGQGTTVTVSS (SEQ ID NO: 13).

The amino acid sequence of the anti-Rh(D) chain D09 is

EVQLLESGGGVVQPGRSLRLSCEASKFTLYNYGMHWVRQAPGKGLEWVAFI

WFDGSNKYYEDSVKGRFTVSRDNSKNTLYLQMNSLRAEDTAVYYCAREGSK

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KVALSRYYYYMDVWGQGTTVTVSS(SEQ ID NO: 14).

The amino acid sequence of the anti-Rh(D) chain D10 is
EVQLLESGGGVVQPGRSLRLSCEASKFTLYNYGMHWVRQAPGKGLEWVAFI
WFDGSNKYYEDSVKGRFTVSRDNSKNTLYLQMNSLRAEDTAVYYCAREVSK

5   KVALSRYYYYMDVWGQGTTVTVSS (SEQ ID NO: 15).

The amino acid sequence of the anti-Rh(D) chain D11 is
EVQLLESGGGVVQPGRSLRLSCEASKFTLYNYGMHWVRQAPGEGLEWVAFIW
FDGSNKYYADSVKGRFTVSRDNSKNTLYLQMNSLRAEDTAVYYCAREVSKKL
ALSRYYYYMDVWGQGTTVTVSS (SEQ ID NO: 16).

10   The amino acid sequence of the anti-Rh(D) chain D12 is
EVQLLESGGGVVQPGRSLRLACAASGFSFRSYGMHWVRQAPGRGLEWVAFT
WFDGSNKYYVDSVKGRFTISRDNSKNTLYLEMNSLRVDDTAVYYCAREASML
RGISRYYYAMDVWGPGTTVTVSS (SEQ ID NO: 17).

The amino acid sequence of the anti-Rh(D) chain D13 is
15   EVQLLESGGGVVQPGRSLRLSCAASGFTFSTYGMHWVRQAPGKGLEWVAVI
WFDGSNRDYAESVKGRFTISRDKSKNTLYLQMNSLRAEDSAVYYCARENVAR
GGGGVRYKYYFDYWGQGTLVTVSS (SEQ ID NO: 18).

The amino acid sequence of the anti-Rh(D) chain D14 is
EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYGMHWVRQAPGKGLEWVAVIW

20   FDGSKRDYAESVKGRFTISRDNSKNTLYLQMNSLRAEDSAVYYCARENVARG
GGGIRYKYYFDYWGQGTLVTVSS (SEQ ID NO: 19).

The amino acid sequence of the anti-Rh(D) chain D15 is
EVQLLESGGGVVQPGRSLRLSCVVSGFTFNNYGMHWVRQAPGKGLEWVAVI
WFDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARENQIK

25   LWSRYLYYFDYWGQGTLVTVSS (SEQ ID NO: 20).

The amino acid sequence of the anti-Rh(D) chain D16 is
EVQLLESGGGVVQPGRSLRLSCVVSGFTFNNYGMHWVRQAPGKGLEWVAVI
WFDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARENQIK
LWSRYLYYFDYWGQGTLVTVSS (SEQ ID NO: 21).

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The amino acid sequence of the anti-Rh(D) chain D17 is
EVQLLESGGGVVQPGRSLRLSCVVSGFTFNNYGMHWVRQAPGKGLEWVAVI
WFDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARENQIK
LWSRYLYYFDYWGQGTLVTVSS (SEQ ID NO: 22).

5   The amino acid sequence of the anti-Rh(D) chain D18 is
EVQLLESGGGVVQPGRSLRLSCVVSGFTFNNYGMHWVRQASGKGLEWVAVI
WFDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARENQIK
LWSRYLYYFDYWGQGTLVTVSS (SEQ ID NO: 23).

The amino acid sequence of the anti-Rh(D) chain D20 is
10   EVQLLESGGGVVQPGRSLRLSCAASGFTFSTYGMHWVRQAPGKGLEWVAVI
WFDGSNKEYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAREEVVR
GVILWSRKFDYWGQGTLVTVSS (SEQ ID NO: 24).

The amino acid sequence of the anti-Rh(D) chain D30 is
EVQLLESGGGVVQPGRSLRLSCAASGFTFSSYGMRWVRQAPGKGLEWVAVV
15   YYDGSNKHYSDSVKGRFTISRDNSKNTLYLQMDSLRAEDTAVYYCARERNFR
SGYSRYYYGMDVWGPGTTVTVSS (SEQ ID NO: 25).

The amino acid sequence of the anti-Rh(D) chain D31 is
EVQLLESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVV
YYDGSNKHYSDSVKGRFTISRDNSKNTLYLQMDSLRAEDTAVYYCARERNFR
20   SGYSRYYYGMDVWGPGTTVTVSS (SEQ ID NO: 26).

The amino acid sequence of the anti-Rh(D) chain E01is
EVQLLESGGGLVKPGGSLRLSCAASGFTFSSYSMHWVRQAPGKGLEWVSSISN
SNTYIYYADAVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARDSRYSNFL
RWVRSDGMDVWGQGTTVIVSS (SEQ ID NO: 27).

25   The amino acid sequence of the anti-Rh(D) chain E03 is
EVQLLESGVESGGGLVKPGGSLRLSCAASGFTFSSYSMHWVRQGPGKGLEWV
SSISNSNTYIYYADAVKGRFTISRDNAKNSLYLQMNSLRAEHTAVYYCARDSR
YSNFLRWVRSDGMDVWGQGTTVIVSS (SEQ ID NO: 28).

The amino acid sequence of the anti-Rh(D) chain F01 is

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AELTQSPSSLSASVGDRVTITCRASQGFRNDLGWYQQKPGKAPKRLIYATSSLQ
SGVPSRFSGSGSGTEFTLTINSLQPEDSATYYCLQHNSFPWTFGQGTKVEIKR
(SEQ ID NO: 29).

The amino acid sequence of the anti-Rh(D) chain G01 is
AELTQSPLSLPVTPGEPASISCRSSQSLLHSSGFNFLDWYLQKPGQSPQLLIYMG
SNRASGVPDRFSGSGSGTDFTLKINRVEAEDVGVYYCMQALQFPLTFGGGTKV
EIKR (SEQ ID NO: 30).

The amino acid sequence of the anti-Rh(D) chain H01 is
AELTQSPSFLSASVGDRVTITCRASQGITSYLAWYQQKPGKAPKLLIYAASTLQ
SGVPSRFSGSGSGTEFTLTIASLQPDDFATYYCQQLNNYPPFTFGPGTKVDIKR
(SEQ ID NO: 31).

The amino acid sequence of the anti-Rh(D) chain I01 is
AELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPYTFGQGTKLEIKR
(SEQ ID NO: 32).

The amino acid sequence of the anti-Rh(D) chain I02 is
AELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTLWTFGQGTKVEIKR
(SEQ ID NO: 33).

The amino acid sequence of the anti-Rh(D) chain I03 is
AELTQSPSSLSASVADRVTITCRTSRNINRYLNWYQQKPGKAPKLLIYAASSLQ
SGVPSRFSGSGSGTDFTLTITSLQPEDFATYYCQQSYSTPFTFGPGTKVDLKR
(SEQ ID NO: 34).

The amino acid sequence of the anti-Rh(D) chain I04 is
AELTQSPSSLSASVGDRVTITCRASQNIRRSLNWYQQKPGKAPKLLIYAASSLQ
SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSSNTPWTFGQGTKVEIKR
(SEQ ID NO: 35).

The amino acid sequence of the anti-Rh(D) chain I05 is
AELTQSPSSLSASVGDRVTITCRASQSIRRYLNWYQHKPGKAPKLLIFAASSLQS

GVPSRFTGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPQTFGQGTKVEIKR
(SEQ ID NO: 36).

The amino acid sequence of the anti-Rh(D) chain I06 is
AELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPITFGQGTRLEIKR (SEQ
ID NO: 37).

The amino acid sequence of the anti-Rh(D) chain I07 is
AELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPRTFGGGTKVEIKR
(SEQ ID NO: 38).

The amino acid sequence of the anti-Rh(D) chain I08 is
AELTQSPFSLSASVGDRVTITCRASQTISRSLNWYQHKPGEAPKLLIYAASSLQR
GVPPRFSGSGSGTDFTLTISSLQPEDFATYFCQQSVRIPYSFGQGTKLEIKR (SEQ
ID NO: 39).

The amino acid sequence of the anti-Rh(D) chain I09 is
AELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQS
GVPSRFSGSGSGTDSTLTISSLQPEDFATYYCQQLNSYPYTFGQGTKLEIKR
(SEQ ID NO: 40).

The amino acid sequence of the anti-Rh(D) chain I10 is
AELTQSPSSLSASVGDRVTITCRASQNISSYLNWYQQKPGKAPKLLIYAASSLQ
SGVLSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPYSFGQGTKLEIKR
(SEQ ID NO: 41).

The amino acid sequence of the anti-Rh(D) chain I11 is
AELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPTLLINAASSLQS
GVPSRFSGSGSGTDFTLTISSLQPEDFAIYYCQQRETFGQGTKLEIKR (SEQ ID
NO: 42).

The amino acid sequence of the anti-Rh(D) chain I12 is
AELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPYTFGQGTKLEIKR

(SEQ ID NO: 43).

The amino acid sequence of the anti-Rh(D) chain I13 is

AELTQSPSSLSASVGDRVTITCRASQSISRYLNWYQQKPGKAPKLLIYAASSLQ

SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYGTPHSFGRGTKLEIKR

5   (SEQ ID NO: 44).

The amino acid sequence of the anti-Rh(D) chain I15 is

AELTQSPSSLSASVGDRVTITCRANQNIRRSLNWYQQKPGKAPNLLIYAASTLQ

GGVPSRFSGSGSGTDFTLTISSLQLADFATYYCQQTSATPWTFGQGTKVEIKR

(SEQ ID NO: 45).

10   The amino acid sequence of the anti-Rh(D) chain I16 is

AELTQSPSSLPASVGDRVTITCRASQTIGFNLNWYQQTSGKPPKLLIYGVSKLQ

NGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQTNDALWTFGQGTKVEVRR

(SEQ ID NO: 46).

The amino acid sequence of the anti-Rh(D) chain J01 is

15 AELQDPVVSVALGQTVRITCQGDGLRSYYASWYQQKPGQAPKLVMYGRNNR

PSGIPGRFSGSSSGQTAALTITGTQAEDEADYYCQSRATSGNPVVFGGGTKLTV

L (SEQ ID NO: 47).

The amino acid sequence of the anti-Rh(D) chain J02 is

AELQDPVVSVALGQTVRITCQGDGLRSYYASWYQQKPGQAPKLVMYGRNNR

20 PSGIPDRFSGSSSGQTAALTITGTQAEDEADYYCQSRATSGNPVVFGGGTKLTV

L (SEQ ID NO: 48).

The amino acid sequence of the anti-Rh(D) chain J04 is

AELQDPVVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNSRPS

GIPDRFSGSSSGNTASLTITGAQAEDEADYYCSSRGSPHVAFGGGTKLTVL

25 (SEQ ID NO: 49).

The amino acid sequence of the anti-Rh(D) chain J05 is

AELQDPVVSVALGQTVKITCQGDSLRKYYASWYQQKPGQAPVLVFYARNSRP

SGIPDRFSGSNSGTTASLTIAGARAEDEADYYCHSRDSNGHHRVFGGGTKLTV

L (SEQ ID NO: 50).

The amino acid sequence of the anti-Rh(D) chain K01 is
AELTQEPSLTVSPGGTVTLTCASSTGAVTSRYFPNWFQQKPGQAPRPLIYSASN
KHSWTPARFSGSLLGGKAALTLSGVQPEDEAEYYCLLYYSGAWVFGGGTKLT
VL (SEQ ID NO: 51).

The amino acid sequence of the anti-Rh(D) chain K02 is
AELTQEPSLTVSPGGTVTLTCASSTGAVTSRYFPNWFQQKPGQAPRPLIYSASN
KHSWTPARFSGSLLGGKAALTLSGVQPEDEAEYYCLLYYSGAWVFGGGTKLT
VL (SEQ ID NO: 52).

The amino acid sequence of the anti-Rh(D) chain K03 is
AELTQPPSLTVSPGGTVTLTCASSTGAVTSRYFPNWFQQKPGQAPRALIYGSNN
KHSWTPARFSGSLLGGKAALTLSGVQPEDEAEYYCLLFYAGAWAFGGWTKLT
VL (SEQ ID NO: 53).

The amino acid sequence of the anti-Rh(D) chain L01 is
AELTQPPSASGTPGQRVTISCSGGSSNIASNTVNWYQQLPGTAPKLLIYSNNQR
PSGVPDRFSGSKSGTSATLVITGLQTGDEADYYCGTWDHSRSGAVFGGGTKLT
VL (SEQ ID NO: 54).

The amino acid sequence of the anti-Rh(D) chain L03 is
AELTQPPSASGTPGQRVTISCSGSSSNIGNNHVSWYQQLPGMAPKLLIYSNGQR
PSGVPDRFSGSKSGTSASLAISGLQSEDEADYYCAAWHDSLYGPVFGGGTKLT
VL (SEQ ID NO: 55).

The amino acid sequence of the anti-Rh(D) chain L04 is
AELTQPPSASGTPGQRVSISCSGSSSNIGSNTVNWYQQLPGTAPKLLISTNNQGP
SGVPDRFSGSKSGTSSSLAISGLRSEAEDDYYCAAWDDTLNGVVFGGGTKLTV
L (SEQ ID NO: 56).

The amino acid sequence of the anti-Rh(D) chain L05 is
AELTQPPSASGTPGLRVTISCSGSSSNIGSNIVNWYQQLPGTAPKLLIFSNNKRPS
GVPDRFSGSKSGTSASLAISGLQSEDEADYYCATWDDSLNGRVFGGGTKLTVL
(SEQ ID NO: 57).

The amino acid sequence of the anti-Rh(D) chain M01 is
AELTQPPSASGTPGQRVTISCSGSNFNIGSNYVFWYQHVPGTAPKLLIYNNQR
PSGVPDRLSGSKSGASASLAINGLRSDDEADYYCTGWDDRLSGLIFGGGPKVT
VL (SEQ ID NO: 58).

5   The amino acid sequence of the anti-Rh(D) chain M02 is
AELTQPPSASGTPGQRVTISCSGSSSNIGSNYVVYWYQQLPGTAPKLLIYRNNQR
PSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCAAWDDSLSGWVFGGGTKLT
VL (SEQ ID NO: 59).

The amino acid sequence of the anti-Rh(D) chain M03 is
10   AELTQPPSASGTPGQRVTISCSGSSSNIGSNYVVYWYQQLPGTAPKLLIYRNNQR
PSGVPDRFSGSKSGTSASLAISGLRSEAEADYYCAAWDDSLSAVVFGGGTKLT
VLL (SEQ ID NO: 60).

The amino acid sequence of the anti-Rh(D) chain N01 is
AELTQPPSVSAAPGQKVTISCSGSSSNIDSNYVSWYQQLPGTAPKLLIFDNYRRP
15   SGIPDRFSGSKSGTSATLGITGLQTGDEADYYCATWDDSLNGRVFGGGTKLTV
L (SEQ ID NO: 61).

The amino acid sequence of the anti-Rh(D) chain N02 is
AELTQPPSVSAAPGQKVTISCSGSSSNIGNNYVSWYQQLPGTAPKLLIYDNNKR
PSGIPDRFSGSKSGTSATLGITGLQTGDEADYYCGTWDSSLSAGRVRRMFGGG
20   TKLTVLG (SEQ ID NO: 62).

The amino acid sequence of the anti-Rh(D) chain O01 is
AELTQPPSVSGAPGQRVTISCTGSSSNIGAPYGVHWYQQFPGTAPKLVIYNDNN
RPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLSGRVFGGGTKLT
VL (SEQ ID NO: 63).

25   The amino acid sequence of the anti-Rh(D) chain O02 is
AELTQPPSVSGAPGQTVTISCTGSSSSIGARYDVHWYQHLPGTAPKLLIYGNHN
RPSGVPDRFSGSKSGTSASLAITGLQAEDEAEYYCQSYDNSLSGSSVFFGGGTK
LTVL (SEQ ID NO: 64).

The amino acid sequence of the anti-Rh(D) chain O03 is

AELTQPPSGAPGQTVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNSRP
SGVPDRFSGSKSGTSASLAITGLQAEDEADYYCQSYDSSLSGPYVVFGGGTKLT
VL (SEQ ID NO: 65).

        The amino acid sequence of the anti-Rh(D) chain P01 is
AELTQPPSVSVAPRQTARITCGGDKIGSNTVHWYRQMSGQAPVLVIYEDKKRP
PGIPERFSGSTSGTTATLSISGAQVEDEADYYCYSRDNSGDQRRVFGAGTKLTV
L (SEQ ID NO: 66).

        The amino acid sequence of the anti-Rh(D) chain Q01 is
AELTQPPSATASLGGSVKLTCILQSGHRNYAVAWHHQEAGKGPRFLMTVTND
GRHIKGDGIPDRFSGSASGAERYLSISGLQSEDEGDYYCQTWGTGMHVFGGGT
KLTVL (SEQ ID NO: 67).

        The amino acid sequence of the anti-Rh(D) chain R01 is
AELTQPPSASGSPGQSVTISCTGASSDVGAYKHVSWYQQHPGKAPKLLTHEGT
KRPSGVPDRFSGSKSGNTASLTVSGLQAEDEADYYCSSFAGNSVIFGGGTKLT
VL (SEQ ID NO: 68).

        The amino acid sequence of the anti-Rh(D) chain S01 is
AELTQPPSVSGSPGQSITISCSDVGNYNLVSWYQQYPGKAPKLIIYEGSKRPSGV
SSRFSGSRSGNTASLTISGLQAEDEADYHCCSYAISSRIFGGGTKLTVL (SEQ ID
NO: 69).

Nucleotide Sequences of Anti-Rh(D) Heavy and Light Chains

        The nucleotide sequence of the portion of the clone encoding the anti-
Rh(D) chain B01 is
GAGGTGCAGCTGCTCGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTC
CCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGGAGCTATGCTATG
CACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGCTAC
AGCATATGATGGAAAAAATAAATACTACGCAGACTCCGTGAAGGGCCGAT
TCACCATCTCCAGAGACAATTCCAAGAACACGCTGTTTCTGCAAATGAACA
GCCTGAGAGCTGAGGACACGGCTGTGTTTTACTGTGCGAGAGGCGGATTTT
ACTATGATAGTAGTGGTTATTACGGCTTGAGGCACTACTTTGACTCCTGGG

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GCCAGGGAACCCTGGTCACCGTCTCCTCA (SEQ ID NO: 70).

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain C01 is

GAGGTGCAGCTGCTCGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTC
CCTGAGACTCTCCTGTGCAGCCTCTGGATTCTCCTTCAGTAGCTATGGCATG
CACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGTCAGTTATA
TCATATGATGGACATCATAAAAACTATGCAGACTCCGTGAAGGGCCGATTC
ACCATCTCCAGAGACAATTCCAAGAAAACGCTGTACCTGCAAATGAACAGC
CTGAGACCTGAGGACACGGCTGTATATTACTGTGCGAACCTAAGGGGGGA
AGTAACTCGTCGTGCGTCTGTTCCCTTTGATATCTGGGGCCCAGGGACAAT
GGTCACCGTCTCTTCA (SEQ ID NO: 71).

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain C03 is

GAGGTGCAGCTGCTCGAGTCGGGGGGAGGTGTGGTCCAGCATGGGAGGTC
CCTGAGACTGTCCTGTGCAGCCTCTGGATTCTCCTTCAGTAGCTATGGCATG
CACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGTCAGTTATA
TCATATGATGGACATCATAAAAACTATGCAGACTCCGTGAAGGGCCGATTC
ACCATCTCCAGAGACAATTCCAAGAAAACGCTGTACCTGCAAATGAACAGC
CTGAGACCTGAGGACACGGCTGTATATTACTGTGCGAACCTAAGGGGGGA
AGTAACTCGTCGTGCGTCTGTTCCCTTTGATATATGGGGCCCAGGGACAAT
GGTCACCGTGTCTTCA (SEQ ID NO: 72).

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain C04 is

GAGGTGCAGCTGCTCGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTC
CCTGAGACTCTCCTGTGCAGCCTCTGGATTCTCCTTCAGTACCTATGGCATG
CACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGTCAGTTATA
TCATATGATGGACATAATAAAAACTATGCAGACTCCGTGAAGGGCCGATTC
ACCATCTCCAGAGACAATTCCAAGAAAACGCTGTACCTGCAAATGAACAGC
CTGAGACCTGAGGACACGGCTGTGTATTACTGTGCGAACCTAAGGGGGGA

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AGTAACTCGTCGTGCGTCTATTCCTTTTGATATCTGGGGCCAAGGGACAAT
GGTCACCGTCTCTTCA (SEQ ID NO: 73).

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain C05 is

GAGGTGCAGCTGCTCGAGTCGGGGGGAGGCGTGGTCCAGCCTGGGAGGTC
CCTGAGACTCTCCTGTGCAGCCTCTGGATTCAGCTTCAGTAGTTATGGCATG
CACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTAT
ATCGTATGATGGAACTAATAAATACTTTGCAGACTCCGTGAAGGGCCGATT
CACCATCTCCAGAGACAATTCCAAGAAAACGCTGTATCTGCAAATGACCAG
CCTGAGACCTGAGGACACGGCTGTGTATTTCTGTGCGAACCTAAGGGGGGA
AGTAACTCGTCGTGCGTCCGTACCTCTTGATATCTGGGGCCAAGGGACAAT
GGTCACCGTCTCTTCA (SEQ ID NO: 74).

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain C08 is

GAGGTGCAGCTGCTCGAGTCGGGGGGAGGCGTGGTCCAGCCTGGGAGGTC
CCTGAGACTCTCCTGTGCAGCCTCTGGATTCAGCTTCAGTAGTTATGGCATG
CACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTAT
ATCGTATGATGGAACTAATAAATACTTTGCAGACTCCGTGAAGGGCCGATT
CACCATCTCCAGAGACAATTCCAAGAAAACGCTGTATCTGCAAATGACCAG
CCTGAGACCTGAGGACACGGCTGTGTATTTCTGTGCGAACCTAAGGGGGGA
AGTAACTCGTCGTGCGTCTGTACCTCTTGATATCTGGGGCCAAGGGACAAT
GGTCACCGTCTCTTCA (SEQ ID NO: 75).

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain C10 is

GAGGTGCAGCTGCTCGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTC
CCTGAGACTCTCCTGTGCAGCCTCTGGATTCTCCTTCAGTAGCTATGGCATG
CACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGTCAGTTATA
TCATATGATGGACATCATAAAACTATGCAGACTCCGTGAAGGGCCGATTC
ACCATCTCCAGAGACAATTCCAAGAAAACGCTGTACCTGCAAATGAACAGC

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CTGAGACCTGAGGACACGGCTGTATATTACTGTGCGAACCTAAGGGGGGA
AGTAACTCGTCGTGCGTCTGTTCCCTTTGATATCTGGGGCCCAGGGACATTG
GTCACCGTCTCTTCA (SEQ ID NO: 76).

The nucleotide sequence of the portion of the clone encoding the anti-
Rh(D) chain D01 is

GAGGTGCAGCTGCTCGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTC
CCTGAGACTCTCCTGTGTAGTGTCTGGTTTCACCTTCAATAACTATGGCATG
CACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATT
TGGTTTGATGGAAGTAATAAATACTATGCAGACTCCGTGAAGGGCCGATTC
ACCATCTCCAGAGACAATTCCAAGAACACACTGTACCTGCAAATGAACAGC
CTGAGAGCCGAGGACACGGCTGTATATTACTGTGCGAGAGAGAACCAGAT
AAAGCTATGGTCCCGATACCTTTACTACTTTGATTACTGGGGCCAGGGAAC
CCTGGTCACCGTCTCCTCA (SEQ ID NO: 77).

The nucleotide sequence of the portion of the clone encoding the anti-
Rh(D) chain D03 is

GAGGTGCAGCTGCTCGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTC
CCTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGTACCTATGGCATG
CACTGGGTCCGCCAGGCTCCAGGCAAGGGACTGGAGTGGGTGGCAGTTAT
ATGGTTTGATGGAAGTAATAAGGAATATGCAGACTCCGTGAAGGGCCGATT
CACCGTCTCCAGAGACAATTCCAAGAACACGCTGTATCTACAAATGAACAG
CCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGAAGAAGTGG
TTCGGGGAGTTATCTTATGGTCTCGGAAGTTTGACTACTGGGGCCAGGGAA
CCCTGGTCACCGTCTCCTCA (SEQ ID NO: 78).

The nucleotide sequence of the portion of the clone encoding the anti-
Rh(D) chain D04 is

GAGGTGCAGCTGCTCGAGTCGGGGGGAGGCGTGGCCCAGCCTGGGAGGTC
CCTGAGACTCTCCTGTGTAGCGTCTGGATTCAGCCTCAGGAGCTATGGCAT
GCACTGGGTCCGCCAGGCTCCTGGCAAGGGGCTGGAGTGGGTGGCAGATA
TATGGTTTGATGGAAGTAATAAAGATTATGCAGACTCCGTGAAGGGCCGAT

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TCACCATCTCCAGAGACAATTCCAAGAACACGTTGTATCTTCAAATGAACA

GCCTGAGAGCCGAGGATACGGCTGTGTATTATTGTGCGAGAGATTGGAGGG

TGCGGGCCTTTAGTAGTGGCTGGTTAAGTGCTTTTGATATCTGGGGCCAAG

GGACAATGGTCACCGTCTCCTCA (SEQ ID NO: 79).

5   The nucleotide sequence of the portion of the clone encoding the anti-

Rh(D) chain D05 is

GAGGTGCAGCTGCTCGAGGAGTCTGGGGGAGGCGTGGCCCAGCCTGGGAG

GTCCCTGAGACTCTCCTGTGTAGCGTCTGGATTCAGCCTCAGGAGCTATGG

CATGCACTGGGTCCGCCAGGCTCCTGGCAAGGGGCTGGAGTGGGTGGCAG

10   ATATATGGTTTGATGGAAGTAATAAAGATTATGCAGACTCCGTGAAGGGCC

GATTCACCATCTCCAGAGACAATTCCAAGAACACGTTGTATCTTCAAATGA

ACAGCCTGAGAGCCGAGGACACGGCTGTGTATTATTGTGCGAGAGATTGGA

GGGTGCGGGCCTTTAGTAGTGGCTGGTTAAGTGCTTTTGATATCTGGGGCC

AAGGGACCACGGTCAGCGTCTCCTCA (SEQ ID NO: 80).

15   The nucleotide sequence of the portion of the clone encoding the anti-

Rh(D) chain D07 is

GAGGTGCAGCTGCTCGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTC

CCTGAGACTCTCCTGTGCAGTGTCTGGATTCACCCTAACTAATTATGGCATG

CACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCACATGT

20   CTGGTATGATGGAAGTAAAACAGAATATGCAGACTCCGTCAAGGGCCGATT

CGCCGTCTCCAGAGACAAATCCAAGAACACACTGTTTCTGCAAATGAACAG

CCTGACAGCCGAGGACACGGCTATTTATTACTGTGCGAGAGAGAGGAGAG

AGAAAGTCTATATATTGTTCTACTCGTGGCTCGACCGCTGGGGCCAGGGAA

CCCTGGTCACCGTCTCCTCA (SEQ ID NO: 81).

25   The nucleotide sequence of the portion of the clone encoding the anti-

Rh(D) chain D08 is

GAGGTGCAGCTGCTCGAGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAG

GTCCCTGAGACTCTCCTGTGCAGCGTCTGGGTTCACCTTCAGTAGCTATGGC

ATGCACTGGGTCCGCCAGGCTCCAGGCAGGGGGCTGGAGTGGGTGGCTCTT

ATATGGTACGATGGAGGTAACAAAGAGTATGCAGACTCCGTGAAGGGCCG
CTTCAGCATCTCCAGAGACAATTCCAAGAACACTCTGTATCTGCAAGTGAA
CAGCCTGAGAGCCGACGACACGGCTGTCTATTACTGTGCGAGAGACCAGA
GAGCAGCAGCGGGTATCTTTTATTATTCCCGTATGGACGTCTGGGGCCAAG
5   GGACCACGGTCACCGTCTCCTCA (SEQ ID NO: 82).

The nucleotide sequence of the portion of the clone encoding the anti-
Rh(D) chain D09 is

GAGGTGCAGCTGCTCGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTC
CCTGAGACTCTCCTGTGAAGCGTCTAAATTCACCCTCTACAATTATGGCATG
10   CACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCATTTATA
TGGTTTGATGGAAGTAATAAATACTATGAAGACTCCGTGAAGGGCCGATTC
ACCGTCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGC
CTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGAAGGATCTAA
GAAGGTGGCACTTTCTAGGTATTACTATTATATGGACGTCTGGGGCCAGGG
15   GACCACGGTCACTGTCTCGTCA (SEQ ID NO: 83).

The nucleotide sequence of the portion of the clone encoding the anti-
Rh(D) chain D10 is

GAGGTGCAGCTGCTCGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTC
CCTGAGACTCTCCTGTGAAGCGTCTAAATTCACCCTCTACAATTATGGCATG
20   CACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCATTTATA
TGGTTTGATGGAAGTAATAAATACTATGAAGACTCCGTGAAGGGCCGATTC
ACCGTCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGC
CTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGAAGTATCTAAG
AAGGTGGCACTTTCTAGGTATTACTACTATATGGACGTCTGGGGCCAGGGG
25   ACCACGGTCACTGTCTCCTCA (SEQ ID NO: 84).

The nucleotide sequence of the portion of the clone encoding the anti-
Rh(D) chain D11 is

GAGGTGCAGCTGCTCGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTC
CCTGAGACTCTCCTGTGAAGCGTCTAAATTCACCCTCTACAATTATGGCATG

CACTGGGTCCGCCAGGCTCCAGGCGAAGGGCTGGAGTGGGTGGCATTTATA
TGGTTTGATGGAAGTAATAAATACTATGCAGACTCCGTGAAGGGCCGATTC
ACCGTCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGC
CTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGAAGTATCTAAG
AAGCTGGCACTTTCTAGGTACTACTACTATATGGACGTCTGGGGCCAGGGG
ACCACGGTCACTGTCTCCTCA (SEQ ID NO: 85).

The nucleotide sequence of the portion of the clone encoding the anti-
Rh(D) chain D12 is

GAGGTGCAGCTGCTCGAGTCGGGGGGAGGCGTGGTCCAGCCTGGGAGGTC
CCTGAGACTCGCCTGTGCAGCGTCTGGATTCAGCTTCAGGAGCTATGGCAT
GCACTGGGTCCGCCAGGCTCCAGGCAGGGGGCTGGAGTGGGTGGCATTTAC
ATGGTTTGATGGAAGCAATAAATATTATGTAGACTCCGTGAAGGGCCGATT
CACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGGAAATGAACAG
CCTGAGAGTCGATGACACGGCTGTATATTACTGTGCGAGAGAGGCGTCTAT
GCTTCGCGGAATTAGCAGATACTACTACGCGATGGACGTCTGGGGCCCAGG
GACCACGGTCACCGTCTCCTCA (SEQ ID NO: 86).

The nucleotide sequence of the portion of the clone encoding the anti-
Rh(D) chain D13 is

GAGGTGCAGCTGCTCGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTC
CCTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGTACTTATGGCATG
CACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTAT
ATGGTTTGATGGAAGTAACAGAGACTATGCAGAGTCCGTGAAGGGCCGATT
CACCATCTCCAGAGACAAGTCCAAGAACACACTGTATCTGCAAATGAACAG
CCTGAGAGCCGAGGACTCGGCTGTGTATTATTGTGCGAGAGAAAATGTGGC
TCGTGGGGGGGGGGGCGTTCGATACAAGTACTACTTTGACTACTGGGGCCA
GGGAACCCTGGTCACCGTCTCCTCA (SEQ ID NO: 87).

The nucleotide sequence of the portion of the clone encoding the anti-
Rh(D) chain D14 is

GAGGTGCAGCTGCTCGAGTCGGGGGGAGGCTTGGTACAGCCTGGGGGGTC

CCTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGTACTTATGGCATG
CACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTAT
ATGGTTTGATGGAAGTAAGAGAGACTATGCAGAGTCCGTGAAGGGCCGATT
CACCATCTCCAGAGACAACTCCAAGAACACACTGTATCTGCAAATGAACAG

5   CCTGAGAGCCGAGGACTCGGCTGTGTATTACTGTGCGAGAGAAAATGTGGC
TCGTGGGGGGGGGGGCATTCGATACAAGTACTACTTTGACTACTGGGGCCA
GGGAACCCTGGTCACCGTCTCCTCA (SEQ ID NO: 88).

The nucleotide sequence of the portion of the clone encoding the anti-
Rh(D) chain D15 is

10   GAGGTGCAGCTGCTCGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTC
CCTGAGACTCTCCTGTGTAGTGTCTGGATTCACCTTCAATAACTATGGCATG
CACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATT
TGGTTTGATGGAAGTAATAAATACTATGCAGACTCCGTGAAGGGCCGATTC
ACCATCTCCAGAGACAATTCCAAGAACACACTGTACCTGCAAATGAACAGC

15   CTGAGAGCCGAGGACACGGCTGTATATTACTGTGCGAGAGAGAACCAGAT
AAAGCTATGGTCCCGATACCTTTACTACTTTGACTACTGGGGCCAGGGAAC
CCTGGTCACCGTCTCCTCA (SEQ ID NO: 89).

The nucleotide sequence of the portion of the clone encoding the anti-
Rh(D) chain D16 is

20   GAGGTGCAGCTGCTCGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTC
CCTGAGACTCTCCTGTGTAGTGTCTGGTTTCACCTTCAATAACTATGGCATG
CACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATT
TGGTTTGATGGAAGTAATAAATACTATGCAGACTCCGTGAAGGGCCGATTC
ACCATCTCCAGAGACAATTCCAAGAACACACTGTACCTGCAAATGAACAGC

25   CTGAGAGCCGAGGACACGGCTGTATATTACTGTGCGAGAGAGAACCAGAT
AAAGCTATGGTCCCGATACCTTTACTACTTTGACTACTGGGGCCAGGGAAC
CCTGGTCACCGTCTCCTCA (SEQ ID NO: 90).

The nucleotide sequence of the portion of the clone encoding the anti-
Rh(D) chain D17 is

GAGGTGCAGCTGCTCGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTC

CCTGAGACTCTCCTGTGTAGTGTCTGGTTTCACCTTCAATAACTATGGCATG

CACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTATT

TGGTTTGATGGAAGTAATAAATACTATGCAGACTCCGTGAAGGGCCGATTC

5   ACCATCTCCAGAGACAATTCCAAGAACACACTGTACCTGCAAATGAACAGC

CTGAGAGCCGAGGACACGGCTGTATATTACTGTGCGAGAGAGAACCAGAT

AAAGCTATGGTCCCGATACCTTTACTACTTTGACTACTGGGGCCAGGGAAC

CCTGGTCACCGTCTCCTCC (SEQ ID NO: 91).

The nucleotide sequence of the portion of the clone encoding the anti-

10   Rh(D) chain D18 is

GAGGTGCAGCTGCTCGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTC

CCTGAGACTCTCCTGTGTAGTGTCTGGTTTCACCTTCAATAACTATGGCATG

CACTGGGTCCGCCAGGCTTCAGGCAAGGGGTTGGAGTGGGTGGCAGTTATT

TGGTTTGATGGAAGTAATAAATACTATGCAGACTCCGTGAAGGGCCGATTC

15   ACCATCTCCAGAGACAATTCCAAGAACACACTGTACCTGCAAATGAACAGC

CTGAGAGCCGAGGACACGGCTGTATATTACTGTGCGAGAGAGAACCAGAT

AAAGCTATGGTCCCGATACCTTTACTACTTTGACTACTGGGGCCAGGGAAC

CCTGGTCACCGTGTCCTCA (SEQ ID NO: 92).

The nucleotide sequence of the portion of the clone encoding the anti-

20   Rh(D) chain D20 is

GAGGTGCAGCTGCTCGAGTCGGGGGGAGGCGTGGTCCAGCCTGGGAGGTC

CCTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGTACCTATGGCATG

CACTGGGTCCGCCAGGCTCCAGGCAAGGGACTGGAGTGGGTGGCAGTTAT

ATGGTTTGATGGAAGTAATAAGGAATATGCAGACTCCGTGAAGGGCCGATT

25   CACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTACAAATGAACAG

CCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGAAGAAGTGG

TTCGGGGAGTTATCTTATGGTCTCGGAAGTTTGACTACTGGGGCCAGGGAA

CCCTGGTCACCGTCTCCTCA (SEQ ID NO: 93).

The nucleotide sequence of the portion of the clone encoding the anti-

Rh(D) chain D30 is

GAGGTGCAGCTGCTCGAGTCGGGGGGAGGCGTGGTCCAGCCTGGGAGGTC
CCTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGTAGCTATGGCATG
CGCTGGGTCCGGCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTGT

5   CTACTATGATGGAAGTAACAAACACTATTCAGACTCCGTGAAGGGCCGATT
CACCATCTCCAGAGACAACTCCAAGAACACGCTGTATCTACAAATGGACAG
CCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGAAAGAAATTT
TCGGAGTGGTTATTCCCGCTACTACTACGGTATGGACGTCTGGGGCCCAGG
GACCACGGTCACCGTCTCCTCA (SEQ ID NO: 94).

10   The nucleotide sequence of the portion of the clone encoding the anti-
Rh(D) chain D31 is

GAGGTGCAGCTGCTCGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTC
CCTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGTAGCTATGGCATG
CACTGGGTCCGGCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTGT

15   CTACTATGATGGAAGTAACAAACACTATTCAGACTCCGTGAAGGGCCGATT
CACCATCTCCAGAGACAACTCCAAGAACACGCTGTATCTACAAATGGACAG
CCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGAAAGAAATTT
TCGGAGTGGTTATTCCCGCTACTACTACGGTATGGACGTCTGGGGCCCAGG
GACCACGGTCACCGTCTCCTCA (SEQ ID NO: 95).

20   The nucleotide sequence of the portion of the clone encoding the anti-
Rh(D) chain E01 is

GAGGTGCAGCTGCTCGAGTCTGGGGGAGGCCTGGTCAAGCCTGGGGGGTC
CCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTAGCTATAGCATG
CACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCATCCATT

25   AGTAATAGTAATACTTACATATACTACGCAGACGCAGTGAAGGGCCGATTC
ACCATCTCCAGAGACAACGCCAAGAACTCACTGTATCTGCAAATGAACAGC
CTGAGAGCCGAGGACACGGCTGTGTACTACTGTGCGAGAGATTCTAGATAC
AGTAATTTCCTCCGTTGGGTTCGGAGCGACGGTATGGACGTCTGGGGCCAA
GGGACCACGGTCATCGTCTCCTCA (SEQ ID NO: 96).

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The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain E03 is

GAGGTGCAGCTGCTCGAGTCTGGGGTGGAGTCTGGGGGAGGCCTGGTCAA

GCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGT

5   AGCTATAGCATGCACTGGGTCCGCCAGGGTCCAGGGAAGGGGCTGGAGTG

GGTCTCATCCATTAGTAATAGTAATACTTACATATACTACGCAGACGCAGT

GAAGGGCCGATTCACCATCTCCAGAGACAACGCCAAGAACTCACTGTATCT

GCAAATGAACAGCCTGAGAGCCGAGCACACGGCTGTGTACTACTGTGCGA

GAGATTCTAGATACAGTAATTTCCTCCGTTGGGTTCGGAGCGACGGTATGG

10   ACGTCTGGGGCCAAGGGACCACGGTCATCGTCTCCTCA (SEQ ID NO: 97).

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain F01 is

GCCGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA

GTCACCATCACTTGCCGGGCAAGTCAGGGCTTTAGAAATGATTTAGGCTGG

15   TATCAGCAGAAACCAGGGAAAGCCCCTAAGCGCCTGATCTATGCTACATCC

AGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGCGGCAGTGGATCTGGGACA

GAATTCACTCTCACAATCAACAGCCTGCAGCCTGAAGATTCTGCAACTTAT

TACTGTCTACAGCATAATAGTTTCCCGTGGACGTTCGGCCAAGGGACCAAG

GTGGAAATCAAACGA (SEQ ID NO: 98).

20   The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain G01 is

GCCGAGCTCACTCAGTCTCCACTCTCCCTGCCCGTCACCCCTGGAGAGCCG

GCCTCCATCTCCTGCAGGTCTAGTCAGAGCCTCCTGCATAGTAGTGGATTCA

ACTTTTTGGATTGGTACCTGCAGAAGCCAGGGCAGTCTCCACAGCTCCTGA

25   TCTATATGGGTTCTAATCGGGCCTCCGGGGTCCCTGACAGGTTCAGTGGCA

GTGGATCAGGCACAGATTTTACACTGAAAATCAACAGAGTGGAGGCTGAG

GATGTTGGGGTTTATTACTGCATGCAAGCTCTACAATTTCCTCTCACTTTCG

GCGGAGGGACCAAGGTGGAGATCAAACGA (SEQ ID NO: 99).

The nucleotide sequence of the portion of the clone encoding the anti-

Rh(D) chain H01 is

GCCGAGCTCACCCAGTCTCCATCCTTCCTGTCTGCATCTGTAGGAGACAGA

GTCACCATCACTTGCCGGGCCAGTCAGGGCATTACGAGTTATTTAGCCTGG

TATCAGCAAAAACCAGGGAAAGCCCCTAAGCTCCTAATCTATGCTGCATCC

ACTTTGCAAAGTGGGGTCCCATCAAGGTTCAGCGGCAGTGGATCTGGGACA

GAATTCACTCTCACAATCGCCAGCCTGCAGCCTGATGATTTTGCAACTTATT

ACTGTCAACAGCTTAATAATTACCCCCCTTTCACTTTCGGCCCTGGGACCAA

AGTGGATATCAAACGA (SEQ ID NO: 100).

The nucleotide sequence of the portion of the clone encoding the anti-
Rh(D) chain I01 is

GCCGAGCTCACCCAGTCTCCATCCTCCCTATCTGCATCTGTAGGAGACAGA

GTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGG

TATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCC

AGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA

GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT

ACTGTCAACAGAGTTACAGTACCCCTCCGTACACTTTTGGCCAGGGGACCA

AGCTGGAGATCAAACGA (SEQ ID NO: 101).

The nucleotide sequence of the portion of the clone encoding the anti-
Rh(D) chain I02 is

GCCGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA

GTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGG

TATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCC

AGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA

GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT

ACTGTCAACAGAGTTACAGTACCCTGTGGACGTTCGGCCAAGGGACCAAGG

TGGAAATCAAACGA (SEQ ID NO: 102).

The nucleotide sequence of the portion of the clone encoding the anti-
Rh(D) chain I03 is

GCCGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGCGGACAGA

GTCACCATCACTTGCCGGACAAGTCGGAACATTAACAGATACTTAAATTGG

TATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATTTATGCTGCATCC

AGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA

GATTTCACTCTCACCATCACCAGTCTGCAACCTGAAGATTTTGCCACTTACT

5   ACTGTCAACAGAGTTACAGTACCCCTTTCACTTTCGGCCCTGGGACCAAAG

TGGATCTCAAACGA (SEQ ID NO: 103).

        The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain I04 is

GCCGAGCTCACTCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA

10   GTCACCATCACTTGCCGGGCAAGTCAGAACATTAGGAGGTCTTTAAATTGG

TATCAACAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCC

AGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA

GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT

ACTGTCAGCAGAGTTCCAATACCCCGTGGACGTTCGGCCAAGGGACCAAGG

15   TGGAAATCAAACGA (SEQ ID NO: 104).

        The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain I05 is

GCCGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA

GTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGGAGGTATTTAAATTGG

20   TATCAGCACAAACCAGGGAAAGCCCCTAAGCTCCTGATCTTTGCTGCATCC

AGTTTGCAAAGTGGGGTCCCATCAAGGTTCACTGGCAGTGGATCTGGGACA

GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT

ACTGTCAACAGAGTTACAGTACCCCTCAAACGTTCGGCCAAGGGACCAAGG

TGGAAATCAAACGA (SEQ ID NO: 105).

25   The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain I06 is

GCCGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA

GTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGG

TATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCCGCATCC

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AGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA

GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT

ACTGTCAACAGAGTTACAGTACCCCGATCACCTTCGGCCAAGGGACACGAC

TGGAGATTAAACGA (SEQ ID NO: 106).

5   The nucleotide sequence of the portion of the clone encoding the anti-

Rh(D) chain I07 is

GCCGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA

GTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGG

TATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCC

10   AGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA

GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT

ACTGTCAACAGAGTTACAGTACCCCTCGAACTTTCGGCGGAGGGACCAAGG

TGGAGATCAAACGA (SEQ ID NO: 107).

The nucleotide sequence of the portion of the clone encoding the anti-

15   Rh(D) chain I08 is

GCCGAGCTCACCCAGTCTCCATTCTCCCTGTCTGCATCTGTCGGAGACAGA

GTCACCATAACTTGCCGGGCAAGTCAGACCATTAGCAGGTCTTTAAATTGG

TATCAGCATAAACCAGGGGAAGCCCCTAAGCTCCTGATCTATGCTGCATCC

AGTCTGCAGCGTGGGGTCCCACCCAGGTTCAGTGGCAGTGGATCTGGGACA

20   GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGACTTTGCGACTTACT

TCTGTCAACAGAGTGTCAGAATCCCGTACAGTTTTGGCCAGGGGACCAAGC

TGGAGATCAAACGA (SEQ ID NO: 108).

The nucleotide sequence of the portion of the clone encoding the anti-

Rh(D) chain I09 is

25   GCCGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA

GTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGG

TATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCC

AGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA

GATTCCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTATT

ACTGTCAACAGCTTAATAGTTACCCGTACACTTTTGGCCAGGGGACCAAGC

TGGAGATCAAACGA (SEQ ID NO: 109).

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain I10 is

GCCGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA

GTCACCATCACTTGCCGGGCAAGTCAGAACATTAGCAGCTATTTAAATTGG

TATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCC

AGTTTGCAAAGTGGGGTCCTATCAAGGTTCAGTGGCAGTGGATCTGGGACA

GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT

ACTGTCAACAGAGTTACAGTACCCCTCCGTATAGTTTTGGCCAGGGGACCA

AGCTGGAGATCAAACGA (SEQ ID NO: 110).

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain I11 is

GCCGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA

GTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGG

TATCAGCAGAAACCAGGGAAAGCCCCTACGCTCCTGATCAATGCTGCATCC

AGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA

GATTTCACTCTCACCATTAGCAGTCTGCAACCTGAAGATTTCGCAATTTACT

ACTGTCAACAGAGAGAAACTTTTGGCCAGGGGACCAAGCTGGAGATCAAA

CGA (SEQ ID NO: 111).

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain I12 is

GCCGAGCTCACCCAGTCTCCATCCTCCCTATCTGCATCTGTAGGAGACAGA

GTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGG

TATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCC

AGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA

GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT

ACTGTCAACAGAGTTACAGTACCCCTCCGTACACTTTTGGCCAGGGGACCA

AGCTGGAGATCAAACGA (SEQ ID NO: 112).

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The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain I13 is

GCCGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCCTCTGTAGGAGACAGA
GTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGGTATTTAAATTGG
TATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCC
AGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA
GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT
ACTGTCAACAGAGTTACGGTACCCCTCACAGTTTTGGCCGGGGGGACCAAGC
TGGAGATCAAACGA (SEQ ID NO: 113).

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain I15 is

GCCGAGCTCACCCAGTCTCCTTCCTCCCTGTCTGCATCTGTAGGAGACAGA
GTCACCATCACTTGCCGGGCAAATCAGAACATTCGTAGATCTTTAAATTGG
TATCAGCAGAAACCAGGGAAAGCCCCTAACCTCCTGATCTATGCTGCATCC
ACATTGCAAGGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA
GATTTCACTCTCACCATCAGCAGTCTGCAACTTGCGGATTTTGCAACTTACT
ACTGTCAACAGACTTCCGCTACCCCGTGGACGTTCGGCCAAGGGACCAAGG
TGGAAATCAAACGA (SEQ ID NO: 114).

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain I16 is

GCCGAGCTCACCCAGTCTCCATCGTCCCTGCCTGCATCTGTGGGAGACAGA
GTCACCATCACTTGCCGGGCAAGTCAGACTATTGGTTTTAATTTAAATTGGT
ATCAGCAAACATCTGGGAAGCCCCCTAAACTCCTAATCTATGGTGTTTCCA
AGTTGCAAAATGGGGTCCCTTCACGGTTCAGTGGCAGTGGGTCCGGGACGG
AATTCACCCTCACAATCAGCAGTCTGCAGCCTGAGGATTTTGCGACTTATTA
TTGTCAACAGACTAACGATGCGTTGTGGACGTTCGGCCAAGGGACCAAAGT
GGAAGTCAGACGA (SEQ ID NO: 115).

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain J01 is

GCCGAGCTCCAGGACCCTGTTGTGTCTGTGGCCTTGGGACAGACAGTCAGG
ATCACTTGCCAAGGAGACGGCCTCAGAAGTTATTATGCAAGCTGGTACCAG
CAGAAGCCGGGACAGGCCCCGAAACTTGTCATGTACGGTAGAAACAACCG
GCCCTCAGGGATCCCAGGCCGATTCTCTGGCTCCAGCTCAGGGCAGACAGC
TGCCTTGACCATCACGGGGACTCAGGCGGAGGATGAGGCTGACTATTACTG
TCAGTCCCGTGCCACCAGCGGTAACCCTGTGGTGTTCGGCGGAGGGACTAA
GCTGACCGTCCTG (SEQ ID NO: 116).

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain J02 is

GCCGAGCTCCAGGACCCTGTTGTGTCTGTGGCCTTGGGACAGACAGTCAGG
ATCACTTGCCAAGGAGACGGCCTCAGAAGTTATTATGCAAGCTGGTACCAG
CAGAAGCCGGGACAGGCCCCGAAACTTGTCATGTACGGTAGAAACAACCG
GCCCTCAGGGATCCCAGACCGATTCTCTGGCTCCAGCTCAGGGCAGACAGC
TGCCTTGACCATCACGGGGACTCAGGCGGAGGATGAGGCTGACTATTACTG
TCAGTCCCGTGCCACCAGCGGTAACCCTGTGGTGTTCGGCGGAGGGACTAA
GCTGACCGTCCTG (SEQ ID NO: 117).

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain J04 is

GCCGAGCTCCAGGACCCTGTTGTGTCTGTGGCCTTGGGACAGACAGTCAGG
ATCACATGCCAAGGAGACAGCCTCAGAAGCTATTATGCAAGCTGGTACCAG
CAGAAGCCAGGACAGGCCCCTGTACTTGTCATCTATGGTAAAAACAGCCGG
CCCTCAGGGATCCCAGACCGATTCTCTGGCTCCAGCTCAGGAAACACAGCT
TCGTTGACCATCACTGGGGCTCAGGCGGAAGATGAGGCGGACTATTATTGT
AGTTCGCGGGGCAGCCCCCACGTGGCATTCGGCGGAGGGACCAAACTGAC
CGTCCTG (SEQ ID NO: 118).

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain J05 is

GCCGAGCTCCAGGACCCTGTTGTGTCTGTGGCCTTGGGACAGACAGTCAAG
ATCACATGCCAGGGAGACAGCCTCAGAAAGTATTATGCAAGCTGGTACCA

GCAGAAGCCAGGACAGGCCCCTGTGCTTGTCTTCTATGCTAGAAATAGCCG

GCCCTCAGGGATCCCAGACCGATTCTCTGGCTCCAACTCAGGAACCACAGC

TTCCTTGACCATCGCTGGGGCTCGGGCGGAAGATGAGGCTGACTATTACTG

TCACTCCCGGGACAGCAATGGTCACCATCGGGTGTTCGGCGGAGGGACCAA

5   GCTGACCGTCCTA (SEQ ID NO: 119).

The nucleotide sequence of the portion of the clone encoding the anti-

Rh(D) chain K01 is

GCCGAGCTCACTCAGGAGCCCTCACTGACTGTGTCCCCAGGAGGGACAGTC

ACTCTCACCTGTGCTTCCAGCACTGGAGCAGTCACCAGTCGTTACTTTCCAA

10   ACTGGTTCCAGCAGAAACCTGGACAAGCACCCAGGCCACTGATTTATAGTG

CAAGCAACAAACACTCCTGGACCCCTGCCCGGTTCTCAGGCTCCCTCCTTG

GGGGCAAAGCTGCCCTGACACTGTCAGGTGTGCAGCCTGAGGACGAGGCT

GAGTATTACTGCCTGCTCTACTATAGTGGTGCTTGGGTGTTCGGCGGAGGG

ACCAAGTTGACCGTCCTT (SEQ ID NO: 120).

15   The nucleotide sequence of the portion of the clone encoding the anti-

Rh(D) chain K02 is

GCCGAGCTCACTCAGGAGCCCTCACTGACTGTGTCCCCAGGAGGGACAGTC

ACTCTCACCTGTGCTTCCAGCACTGGAGCAGTCACCAGTCGTTACTTTCCAA

ACTGGTTCCAGCAGAAACCTGGACAAGCACCCAGGCCACTGATTTATAGTG

20   CAAGCAACAAACACTCCTGGACCCCTGCCCGGTTCTCAGGCTCCCTCCTTG

GGGGCAAAGCTGCCCTGACACTGTCAGGTGTGCAGCCTGAGGACGAGGCT

GAGTATTACTGCCTGCTCTACTATAGTGGTGCTTGGGTGTTCGGCGGAGGG

ACCAAGCTGACCGTCCTA (SEQ ID NO: 121).

The nucleotide sequence of the portion of the clone encoding the anti-

25   Rh(D) chain K03 is

GCCGAGCTCACTCAGCCACCCTCACTGACTGTGTCCCCAGGAGGGACAGTC

ACTCTCACCTGTGCTTCCAGCACTGGAGCAGTCACCAGTCGTTACTTTCCAA

ACTGGTTCCAGCAGAAACCTGGCCAGGCACCCAGGGCACTGATTTATGGTT

CAAACAACAAACACTCCTGGACCCCTGCCCGGTTCTCAGGCTCCCTCCTTG

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GGGGCAAAGCTGCCCTGACACTGTCAGGTGTGCAGCCTGAGGACGAGGCG
GAGTATTACTGCCTGCTCTTCTATGCTGGTGCTTGGGCGTTCGGCGGATGGA
CCAAGCTGACCGTCCTA (SEQ ID NO: 122).

The nucleotide sequence of the portion of the clone encoding the anti-
Rh(D) chain L01 is
GCCGAGCTCACGCAGCCGCCCTCAGCGTCTGGGACCCCCGGGCAGAGGGT
CACCATCTCTTGTTCTGGAGGCAGCTCCAACATCGCAAGTAATACTGTAAA
CTGGTACCAGCAACTCCCAGGAACGGCCCCCAAACTCCTCATCTATAGTAA
TAATCAGCGGCCCTCAGGGGTCCCTGACCGATTCTCTGGCTCCAAGTCTGG
CACCTCAGCCACCCTGGTCATCACCGGGCTCCAGACTGGGGACGAGGCCGA
TTATTACTGCGGAACATGGGATCACAGCCGGAGTGGTGCGGTGTTCGGCGG
AGGGACCAAACTGACCGTCTTA (SEQ ID NO: 123).

The nucleotide sequence of the portion of the clone encoding the anti-
Rh(D) chain L03 is
GCCGAGCTCACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTC
ACCATCTCTTGTTCTGGCAGTAGCTCCAACATCGGAAATAATCATGTAAGC
TGGTACCAGCAACTCCCAGGAATGGCCCCCAAACTCCTCATCTATTCTAAT
GGTCAGCGGCCCTCAGGGGTCCCTGACCGATTCTCTGGCTCCAAGTCTGGC
ACCTCAGCCTCCCTGGCCATCAGCGGCCTCCAGTCTGAGGATGAGGCTGAT
TATTATTGTGCAGCATGGCATGACAGCCTCTATGGTCCGGTGTTCGGCGGA
GGGACCAAGCTGACCGTCCTC (SEQ ID NO: 124).

The nucleotide sequence of the portion of the clone encoding the anti-
Rh(D) chain L04 is
GCCGAGCTCACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTC
AGCATCTCTTGTTCTGGAAGCAGCTCCAACATCGGAAGTAATACTGTAAAC
TGGTACCAGCAGCTCCCAGGAACAGCCCCCAAACTCCTCATCTCTACTAAT
AATCAGGGGCCCTCAGGAGTCCCTGACCGATTCTCTGGCTCCAAGTCTGGC
ACCTCATCCTCCCTGGCCATCAGTGGGCTCCGGTCAGAGGCTGAGGATGAT
TATTACTGTGCAGCATGGGATGACACCCTGAATGGTGTGGTATTCGGCGGA

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GGGACCAAACTGACCGTCCTA (SEQ ID NO: 125).

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain L05 is

GCCGAGCTCACTCAGCCACCCTCAGCGTCTGGGACTCCCGGGCTGAGGGTC

ACCATCTCTTGTTCTGGAAGCAGCTCCAACATCGGAAGTAATATTGTAAAC

TGGTACCAGCAGCTCCCAGGAACGGCCCCCAAACTCCTCATCTTTAGTAAT

AATAAGCGGCCCTCAGGGGTCCCTGACCGATTCTCTGGCTCCAAGTCTGGC

ACCTCAGCCTCCCTGGCCATCAGTGGGCTCCAGTCTGAGGATGAGGCTGAT

TATTACTGTGCTACATGGGATGACAGCCTGAATGGTCGGGTGTTCGGCGGA

GGGACCAAGCTGACCGTCCTA (SEQ ID NO: 126).

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain M01 is

GCCGAGCTCACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGCGGGTC

ACCATCTCTTGTTCTGGGAGCAACTTCAACATCGGAAGTAATTATGTATTCT

GGTACCAGCATGTTCCAGGAACGGCCCCAAAACTCCTCATCTATAATAATA

ATCAACGCCCCTCTGGGGTCCCTGACCGACTCTCTGGCTCCAAGTCTGGCG

CCTCAGCCTCCCTGGCCATCAATGGGCTCCGGTCCGATGATGAGGCTGATT

ATTACTGTACAGGATGGGATGACCGCCTGAGTGGCCTGATTTTCGGCGGAG

GGCCAAAAGTGACCGTCCTA (SEQ ID NO: 127).

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain M02 is

GCCGAGCTCACGCAGCCGCCCTCAGCGTCTGGGACCCCCGGGCAGAGGGT

CACCATCTCTTGTTCTGGAAGCAGCTCCAACATCGGAAGTAATTATGTATAT

TGGTACCAGCAGCTCCCAGGAACGGCCCCCAAACTCCTCATCTATAGGAAT

AATCAGCGGCCCTCAGGGGTCCCTGACCGATTCTCTGGCTCCAAGTCTGGC

ACCTCAGCCTCCCTGGCCATCAGTGGGCTCCGGTCCGAGGATGAGGCTGAT

TATTACTGTGCAGCATGGGATGACAGCCTGAGTGGTTGGGTGTTCGGCGGA

GGGACCAAGCTGACCGTCCTA (SEQ ID NO: 128).

The nucleotide sequence of the portion of the clone encoding the anti-

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Rh(D) chain M03 is

GCCGAGCTCACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTC
ACCATCTCTTGTTCTGGAAGCAGCTCCAACATCGGAAGTAATTATGTATACT
GGTACCAGCAGCTCCCAGGAACGGCCCCCAAACTCCTCATCTATAGGAATA
ATCAGCGGCCCTCAGGGGTCCCTGACCGATTCTCTGGCTCCAAGTCTGGCA
CCTCAGCCTCCCTGGCCATCAGTGGGCTCCGGTCCGAGGCTGAGGCTGATT
ATTACTGTGCGGCATGGGATGACAGCCTGAGTGCCGTGGTATTCGGCGGAG
GGACCAAACTGACCGTCCTA (SEQ ID NO: 129).

5

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain N01 is

10

GCCGAGCTCACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAAGGTC
ACCATCTCCTGCTCTGGAAGCAGCTCCAACATTGACAGTAACTATGTATCCT
GGTACCAGCAGCTCCCAGGAACAGCCCCCAAACTCCTCATTTTTGACAATT
ATAGGCGACCCTCAGGGATTCCTGACCGATTCTCAGGCTCCAAGTCTGGCA
CGTCAGCCACCCTGGGCATCACCGGACTCCAGACTGGGGACGAGGCCGATT
ATTACTGTGCAACATGGGATGACAGCCTGAATGGTCGGGTGTTCGGCGGAG
GGACCAAGCTGACCGTCCTA (SEQ ID NO: 130).

15

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain N02 is

20

GCCGAGCTCACGCAGCCGCCCTCAGTGTCTGCGGCCCCAGGACAGAAGGTC
ACCATCTCCTGCTCTGGAAGCAGCTCCAACATTGGGAATAATTATGTGTCCT
GGTACCAGCAACTCCCAGGAACAGCCCCCAAACTCCTCATTTATGACAATA
ATAAGCGACCCTCAGGGATTCCTGACCGATTCTCTGGCTCCAAGTCTGGCA
CGTCAGCCACCCTGGGCATCACCGGACTCCAGACTGGGGACGAGGCCGATT
ATTACTGCGGAACATGGGATAGCAGCCTGAGTGCTGGCCGCGTTCGGCGGA
TGTTCGGCGGAGGGACCAAGTTGACCGTCCTGGGT (SEQ ID NO: 131).

25

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain O01 is

GCCGAGCTCACGCAGCCGCCCTCAGTGTCTGGGGCCCCAGGGCAGAGGGT

CACCATCTCCTGCACTGGGAGCAGCTCCAACATCGGGGCACCTTATGGTGT

ACACTGGTACCAGCAGTTTCCAGGAACAGCCCCCAAACTCGTCATCTACAA

TGACAACAATCGGCCCTCAGGGGTCCCTGACCGATTCTCTGGCTCCAAGTC

TGGCACCTCAGCCTCCCTGGCCATCACTGGGCTCCAGGCTGAGGATGAGGC

5   TGATTATTACTGCCAGTCCTATGACAGCAGCCTGAGTGGAAGGGTGTTCGG

CGGAGGGACCAAGCTGACCGTCCTA (SEQ ID NO: 132).

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain O02 is

GCCGAGCTCACGCAGCCGCCCTCAGTGTCTGGGGCCCCAGGGCAGACGGTC

10   ACCATCTCCTGCACTGGGAGCAGCTCCAGCATCGGGGCACGTTATGATGTA

CACTGGTACCAACACCTTCCAGGAACAGCCCCCAAACTCCTCATCTATGGT

AACCACAATCGGCCCTCAGGGGTCCCTGACCGATTCTCTGGCTCCAAGTCT

GGCACCTCAGCCTCCCTGGCCATCACTGGGCTCCAGGCTGAGGATGAGGCT

GAATATTATTGCCAGTCCTATGACAACAGCCTGAGTGGTTCGTCTGTCTTTT

15   TCGGCGGAGGGACCAAGCTGACCGTCCTA (SEQ ID NO: 133).

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain O03 is

GCCGAGCTCACGCAGCCGCCCTCTGGGGCCCCAGGCCAGACGGTCACCATC

TCCTGCACTGGGAGCAGCTCCAACATCGGGGCAGGTTATGATGTACACTGG

20   TACCAGCAGCTTCCAGGAACAGCCCCCAAACTCCTCATCTATGGTAACAGC

AATCGGCCCTCAGGGGTCCCTGACCGATTCTCTGGCTCCAAGTCTGGCACC

TCAGCCTCCCTGGCCATCACTGGGCTCCAGGCTGAGGATGAGGCTGATTAT

TACTGCCAGTCCTATGACAGCAGCCTGAGTGGTCCCTATGTGGTATTCGGC

GGAGGGACCAAGCTGACCGTCCTA (SEQ ID NO: 134).

25   The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain P01 is

GCCGAGCTCACTCAGCCACCCTCGGTGTCAGTGGCCCCAAGACAGACGGCC

AGGATTACCTGTGGGGGGGACAAAATCGGAAGTAACACTGTGCATTGGTA

CCGGCAGATGTCAGGCCAGGCCCCTGTTCTGGTCATCTATGAAGACAAAAA

ACGACCCCCCGGGATCCCTGAGAGATTCTCTGGTTCCACCTCAGGGACAAC

GGCCACCTTGAGTATCAGTGGGGCCCAGGTTGAGGATGAAGCTGACTACTA

CTGTTATTCAAGAGACAACAGTGGTGATCAGAGAAGGGTGTTCGGCGCAG

GGACCAAGCTGACCGTCCTA (SEQ ID NO: 135).

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain Q01 is

GCCGAGCTCACTCAGCCACCCTCCGCCACTGCCTCCCTGGGAGGCTCGGTC

AAACTCACCTGCATTCTGCAGAGTGGCCACAGAAATTACGCCGTCGCTTGG

CATCACCAAGAAGCAGGGAAGGGCCCGCGATTTTTGATGACGGTTACCAAT

GATGGCAGGCACATCAAGGGGGACGGGATCCCTGATCGCTTCTCAGGCTCC

GCCTCTGGGGCTGAACGCTACCTCTCCATCTCCGGCCTCCAGTCTGAGGAT

GAGGGTGACTACTACTGTCAGACCTGGGGCACTGGCATGCATGTGTTCGGC

GGAGGGACCAAACTGACCGTCCTA (SEQ ID NO: 136).

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain R01 is

GCCGAGCTCACTCAGCCTCCCTCCGCGTCCGGGTCTCCTGGACAGTCAGTC

ACCATCTCCTGCACTGGAGCCAGCAGTGACGTTGGTGCTTATAAGCACGTC

TCCTGGTACCAACAACACCCAGGCAAAGCCCCCAAACTCCTGACTCATGAG

GGCACTAAGCGGCCCTCAGGGGTCCCTGATCGCTTCTCTGGCTCCAAGTCT

GGCAACACGGCCTCCCTGACCGTCTCTGGGCTCCAGGCTGAGGATGAGGCT

GATTATTACTGCAGCTCATTTGCAGGTAATTCCGTGATATTCGGCGGAGGG

ACCAAGCTGACCGTCCTA (SEQ ID NO: 137).

The nucleotide sequence of the portion of the clone encoding the anti-Rh(D) chain S01 is

GCCGAGCTCACTCAGCCTCCCTCCGTGTCTGGGTCTCCTGGACAGTCGATC

ACCATCTCCTGCAGTGATGTTGGGAATTATAACCTTGTCTCCTGGTACCAAC

AGTACCCAGGCAAGGCCCCCAAACTCATAATTTATGAGGGCAGTAAGCGG

CCCTCAGGGGTTTCTAGTCGCTTCTCTGGCTCCAGGTCTGGCAACACGGCCT

CCCTGACAATCTCTGGGCTCCAGGCTGAGGACGAGGCTGATTATCACTGCT

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GCTCATATGCAATTAGTAGCAGGATTTTCGGCGGAGGGACCAAGCTGACCG
TCCTA (SEQ ID NO: 138).

<div align="center">Example 3</div>

<div align="center">Isolation of Anti-Rh(D) Monoclonal Antibodies</div>

<div align="center">to Conventional and Novel Epitopes</div>

<div align="center">Using a Heavy Chain/Light Chain Shuffling Approach</div>

In view of the results obtained in Examples 1 and 2 herein, heavy and
light chains of antibodies of various Rh(D) epitope specificities were randomly
recombined in order to generate anti-Rh(D) antibodies having additional patterns of
reactivity with Rh(D) variant cells. Using this approach, plasmid DNA obtained from
the Fab/phage display libraries described in panning rounds 2 and 3 of Example 1 was
randomly recombined to generate a "shuffled" Fab/phage display library. When the
Rh(D) specificity of antibodies of this "shuffled" library was determine, it was found
that many of these antibodies exhibited novel epitope specificity. Significantly,
antibody clones having novel Rh(D) epitope specificity were identified, including
clones which bind to wild type and certain partial D type red blood cells but which do
not bind to D category III red blood cells. The experiments described in this Example
therefore demonstrate that the methods described in this specification may be used to
generate antibody clones useful for diagnostic and therapeutic applications in humans.

The materials and methods used in the experiments described in this
Example are now described.

Creation of Shuffled Fab/Phage Display Library

Two microgram aliquots of DNA obtained from libraries LP2, LP3,
KP2, and KP3 (described herein in Example 1) were digested using the restriction
endonucleases *Spe*I and *Xho*I (15 and 60 units, respectively) in order to dissociate DNA
segments encoding individual (full length) heavy chains from library plasmids
encoding individual (full length) light chains. Endonuclease/DNA mixtures were
incubated overnight at 37°C. After the restriction endonucleases were removed using

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standard phenol/chloroform and chloroform extraction techniques, the DNA was precipitated using ethanol.

Equivalent amounts of DNA from each of the four libraries (500 nanograms total) were mixed, and then the heavy chain-encoding DNA fragments were re-ligated into the library plasmids encoding individual light chains. This ligation was performed overnight at 20°C in the presence of 3.5 units of T4 DNA ligase in a total reaction volume of 70 microliters. This treatment generated re-ligated library plasmids encoding a light chain and a heavy chain, wherein the light chain and the heavy chain were not necessarily encoded by a single plasmid in the original library DNA. For this reason, the library of re-ligated plasmids was designated a "shuffled" library.

Three microliters of shuffled library suspension were mixed with an aliquot of XL1-Blue electrocompetent cells (obtained from Stratagene, La Jolla, CA), and the cells were electroporated according to standard methods. Electroporated cells were cultured on plates containing Luria broth comprising 100 micrograms per milliliter carbenicillin.

Anti-Rh(D) Specificity of "Shuffled" Library Antibodies

Fifty-six randomly chosen colonies were selected, and monoclonal Fab/phage preparations were separately produced from each of these individual colonies, using the methods described herein in Example 1. Rh(D) specificity was determined by indirect agglutination using anti-M13 antibody, as described herein in Examples 1 and 2. Plasmid DNA was separately prepared from each of the Fab/phage preparations which exhibited Rh(D) specificity, and the DNA sequences encoding the heavy and light chains expressed by each preparation were determined as described herein.

The results of the experiments presented in this Example are now described.

Anti-Rh(D) Specificity of "Shuffled" Library Antibodies

Of the 56 randomly-chosen "shuffled" library clones, 34 (61%) demonstrated specificity for Rh(D). The Rh(D) epitope specificity, the agglutination

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pattern, and the heavy and light chain sequences of these 34 clones are listed in Table 4. Of these 34 clones, 19 exhibited specificity for previously-described Rh(D) epitopes (e.g. epD 1, epD 2, epD 6/7, and epD X), and one bound too weakly to wild-type Rh(D)-positive red blood cells to characterize is epitope specificity (i.e. clone SH44). However, 14 of the clones identified in Table 4 exhibited novel Rh(D) epitope specificity. Some of these 14 antibody clones comprised a heavy chain, a light chain, or both, that were identified herein in Examples 1 or 2. However, half (17/34) of the heavy chain sequences and about 80% (28/34) of the light chain sequences had not been identified in Examples 1 or 2.

The Rh(D)-specific antibody clones isolated from the "shuffled" library are useful for characterizing and classifying patient red blood cells that express variant forms of the Rh(D) antigen. Of particular interest are clones SH18, SH20, and SH46. These three clones agglutinate wild type red blood cells and certain partial D-type red blood cells, but do not agglutinate D category III red blood cells (a.k.a. partial Rh(D)III cells). It is believed that all previously-characterized human monoclonal anti-Rh(D) antibodies agglutinate D category III red blood cells. Therefore these three clones are particularly useful for differentiating D category III red blood cells from other types of red blood cells.

From a clinical perspective, it has heretofore only been possible to retrospectively identify D category III red blood cells in a patient after they have been erroneously presumed to have wild-type Rh(D)-positive cells. For example, transfusion of an individual having D category III red blood cells with wild-type Rh(D) cells induces production of anti-Rh(D) alloantibodies in the individual. Previously, the presence of D category III red blood cells in patients could only be determined by the production of such anti-Rh(D) alloantibodies in a transfusion recipient who does not naturally harbor D category III red blood cells. Although providing transfused blood comprising D category III red blood cells to a patient who does not naturally harbor such cells will not necessarily cause immediate harm to the patient, the patient thereby becomes alloimmunized against D category III red blood cells. Such alloimmunized

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individuals may develop complications including hemolytic transfusion reactions or hemolytic disease of the newborn.

Table 4. Analysis of Anti-RH(D) Clones Obtained by Chain Shuffling.

CLONE	HEAVY CHAIN SEQUENCE †	LIGHT CHAIN SEQUENCE †	AGGLUTINATION PATTERN ‡							Rh(D) SPECIFICITY
			wt	III	IVa	IVb	V	V1	VII	
SH04	SEQ ID NOs: 24/93	SEQ ID NOs: 35/104	+	+	+	+	+	0	+	epD 6/7
SH08	SEQ ID NOs: 12/81	SEQ ID NOs: 154/197	+	+	+	+	+	0	+	epD 6/7
SH10	SEQ ID NOs: 139/182	SEQ ID NOs: 47/116	+	0	0	0	0	0	0	novel
SH12	SEQ ID NOs: 9/78	SEQ ID NOs: 155/198	+	+	+	+	+	0	+	epD 6/7
SH13	SEQ ID NOs: 26/95	SEQ ID NOs: 156/199	+	0	0	0	0	0	0	novel
SH14	SEQ ID NOs: 24/93	SEQ ID NOs: 157/200	+	+	+	+	+	0	+	epD 6/7
SH16	SEQ ID NOs: 140/183	SEQ ID NOs: 158/201	+	0	+	+	0	0	0	novel
SH17	SEQ ID NOs: 141/184	SEQ ID NOs: 47/116	+	+	0	0	0	0	+	epD 1
SH18	SEQ ID NOs: 142/185	SEQ ID NOs: 159/202	+	0	+	+	0	0	0	novel
SH20	SEQ ID NOs: 143/186	SEQ ID NOs: 160/203	+	0	+	+	+	0	0	novel
SH21	SEQ ID NOs: 9/78	SEQ ID NOs: 161/204	+	+	+	0	+	0	0	novel
SH24	SEQ ID NOs: 144/187	SEQ ID NOs: 162/205	+	0	0	0	0	0	0	novel
SH25	SEQ ID NOs: 145/188	SEQ ID NOs: 35/104	+	+	0	0	+	0	+	epD 2
SH26	SEQ ID NOs: 21/90	SEQ ID NOs: 163/206	+	+	+	0	0	0	0	novel
SH28	SEQ ID NOs: 146/189	SEQ ID NOs: 164/207	+	+	0	0	+	0	+	epD2

CLONE	HEAVY CHAIN SEQUENCE †	LIGHT CHAIN SEQUENCE †	AGGLUTINATION PATTERN ‡							Rh(D) SPECIFICITY
			wt	III	IVa	IVb	V	V1	VII	
SH30	SEQ ID NOs: 12/81	SEQ ID NOs: 165/208	+	+	+	+	+	0	+	epD 6/7
SH32	SEQ ID NOs: 147/190	SEQ ID NOs: 166/209	+	0	0	0	0	0	0	novel
SH34	SEQ ID NOs: 5/74	SEQ ID NOs: 167/210	+	+	0	0	0	0	+	epD 1
SH36	SEQ ID NOs: 14/83	SEQ ID NOs: 168/211	+	0	0	0	0	0	0	novel
SH37	SEQ ID NOs: 148/191	SEQ ID NOs: 50/119	+	+	+	0	0	0	+	epD X §
SH39	SEQ ID NOs: 149/192	SEQ ID NOs: 169/212	+	0	0	0	0	0	0	novel
SH41	SEQ ID NOs: 24/93	SEQ ID NOs: 170/213	+	+	+	+	+	0	+	epD 6/7
SH44	SEQ ID NOs: 150/193	SEQ ID NOs: 171/214	w*							not determined
SH46	SEQ ID NOs: 13/82	SEQ ID NOs: 172/215	+	0	+	+	0	0	0	novel
SH47	SEQ ID NOs: 151/194	SEQ ID NOs: 173/216	+	+	0	0	+	0	+	epD 2
SH48	SEQ ID NOs: 6/75	SEQ ID NOs: 174/217	+	+	0	0	0	0	+	epD 1
SH49	SEQ ID NOs: 17/86	SEQ ID NOs: 175/218	+	+	0	0	0	0	+	epD 1
SH50	SEQ ID NOs: 146/189	SEQ ID NOs: 176/219	+	+	0	0	0	0	+	epD 1
SH51	SEQ ID NOs: 17/86	SEQ ID NOs: 177/220	+	+	0	0	+	0	+	epD 2
SH52	SEQ ID NOs: 24/93	SEQ ID NOs: 178/221	+	+	0	0	0	0	+	epD 1
SH53	SEQ ID NOs: 146/189	SEQ ID NOs: 47/116	+	0	0	0	0	0	0	novel

CLONE	HEAVY CHAIN SEQUENCE †	LIGHT CHAIN SEQUENCE †	AGGLUTINATION PATTERN ‡							Rh(D) SPECIFICITY
			wt	III	IVa	IVb	V	VI	VII	
SH54	SEQ ID NOs: 152/195	SEQ ID NOs: 179/222	+	+	0	0	0	0	+	epD 1
SH55	SEQ ID NOs: 21/90	SEQ ID NOs: 180/223	+	+	0	0	+	0	+	epD 2
SH56	SEQ ID NOs: 153/196	SEQ ID NOs: 181/224	+	+	0	0	0	0	0	novel

Notes for Table 4

† "SEQ ID NOs: A/B" means that the chain had amino acid sequence "A" and was encoded by nucleotide sequence "B".

‡ "+" means agglutination occurred; "0" means agglutination did not occur.

* weak

§ as discussed in Example 2.

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Amino Acid Sequences of Anti-Rh(D) Heavy and Light Chains

The amino acid sequences of various anti-Rh(D) antibody chains were as follows, and are represented using single letter amino acid codes.

The amino acid sequence of the heavy chain of anti-Rh(D) antibody clone SH10 is

EVQLLEESGGGVVQPGRSLRLSCAASGFTFSRNGMHWVRQAPGKGLEWVAFI
WFDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRADDTAVYYCAREEALF
RGLTRWSYGMDVWGQGTTVSVSS (SEQ ID NO: 139).

The amino acid sequence of the heavy chain of anti-Rh(D) antibody clone SH16 is

EVQLLESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGRGLEWVALIW
YDGGNKEYADSVKGRFSISRDNSKNTLYLQVNSLRADDTAVYYCARDQRAA
AGIFYYSRMDVWGQGTTVTVSS (SEQ ID NO: 140).

The amino acid sequence of the heavy chain of anti-Rh(D) antibody clone SH17 is

EVQLLESGGGLVQPGGSLRLSCGASGIPFVSSWMAWVRQAPGKGLEWVANIK
QDGSKKNYVDSVEGRFTISRDNAKNSLYLQMDSLRAEDTRIYYCARDSLTCFD
YWGQGALVTVSS (SEQ ID NO: 141).

The amino acid sequence of the heavy chain of anti-Rh(D) antibody clone SH18 is

EVQLLESGGGVVQPGRSLRLSCAASGFTFRSYAMHWVRQAPGKGLEWVAAT
AYDGKNKYYADSVKGRFTISRDNSMNTLFLQMNSLRAEDTAVFYCARGGFYY
DSSGYYGLRHYFDSWGQGTLVTVSS (SEQ ID NO: 142).

The amino acid sequence of the heavy chain of anti-Rh(D) antibody clone SH20 is

EVQLLEESGGGVVQPGRSLRLSCAASGFTFRSYAMHWVRQAPGKGLEWVAVI
SYDGSTIYYADSVKGRFTISRANSKNTLFLQMNSLRTEDTAVYYCTRGGFYYD
SSGYYGLRHYFDYWGQGTLVTVSS (SEQ ID NO: 143).

The amino acid sequence of the heavy chain of anti-Rh(D) antibody clone SH24 is

EVQLLESGGGVAQPGRSLRLSCVASGFSLRSYGMHWVRQAPGKGLEWVADI WFDGSNKDYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDWRV RAFSSGWLSAFDIWGQGTMVTVSS (SEQ ID NO: 144).

The amino acid sequence of the heavy chain of anti-Rh(D) antibody clone SH25

EVQLLEESGGGVVQPGRSLRLACAASGFSFRSYGMHWVRQAPGRGLEWVAFT WFDGSNKYYVDSVKGRFTISRDNSKNTLYLEMNSLRVDDTAVYYCAREAPML RGISRYYYAMDVWGPGTTVTVSS (SEQ ID NO: 145).

The amino acid sequence of the heavy chain of each of anti-Rh(D) antibody clones SH28, SH50, and SH53 is

EVQLLESGGGGVQPGRSLRLSCAASGFTFNSYAMYWVRQPPGKGLEWVAAIW YDGSNKEYADFVKGRFTISRDNSKNTLSLQMNSLRDEDTAVYYCAREANLLR GWSRYYYGMDVWGQGTTVTVSS (SEQ ID NO: 146).

The amino acid sequence of the heavy chain of anti-Rh(D) antibody clone SH32 is

EVQLLESGGGVVQPGRSLRLSCEASKFTLYNYGMHWVRQAPGKGLEWVAFI WFDGSNKYYEDSVKGRFTVSRDNSKNTLYLQMNSLRAEDTAVYYCARELSK KVALSRYYYYMDVWGQGTTVTVSS (SEQ ID NO: 147).

The amino acid sequence of the heavy chain of anti-Rh(D) antibody clone SH37 is

EVQLLESGGGVVQPGRSLRLSCEASKFTLYNYGMHWVRQAPGKGLEWVAFI WFDGSNKYYEDSVKGRFTVSRDNSKNTLYLQMNSLRAEDTAVYYCARELSK KVALSRYYYYMDVWGQGTTVTVSS (SEQ ID NO: 148).

The amino acid sequence of the heavy chain of anti-Rh(D) antibody clone SH39 is

EVQLLEQSGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVI

WFDGSNKEYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAREEVVR
GVILWSRKFDYWGQGTLVTVSS (SEQ ID NO: 149).

The amino acid sequence of the heavy chain of anti-Rh(D) antibody
clone SH44 is

5   EVQLLESGGGVAQPGRSLRLSCVASGFSLRSYGMHWVRQAPGKGLEWVADI
WFDGSNKDYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDWRV
RAFSSGWLSAFDIWGQGTMVTVSS (SEQ ID NO: 150).

The amino acid sequence of the heavy chain of anti-Rh(D) antibody
clone SH47 is

10   EVQLLESGGGVVQPGRSLRLSCAASGFSFSNYAMHWVRQAPGKGLEWVAVTS
FDGSIKDYADSVKGRFTISRDNSKNTLYLQMNSLRDEDTAVYYCARERGMIVV
VRRRNAFDIWGQGTMVTVSS (SEQ ID NO: 151).

The amino acid sequence of the heavy chain of anti-Rh(D) antibody
clone SH54 is

15   EVQLLESGGGVVQPGRSLRLSCAASGFTFSRNGMHWVRQAPGKGLEWVAFIW
FDGSNKYYADSVKGRFTISRDNSKNTLYLQMNSLRADDTAVYYCAREEALFR
GLTRWSYGMDVWGQGTTVSVSS (SEQ ID NO: 152).

The amino acid sequence of the heavy chain of anti-Rh(D) antibody
clone SH56 is

20   EVQLLESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKGLEWVAVV
YYDGSNKHYSDSVKGRFTIFRDNSKNTLYLQMDSLRAEDTAVYYCARERNFR
SGYSRYYYGMDVWGPGTTVTVSS (SEQ ID NO: 153).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone
SH8 is

25   AELTQSPSSLAASVGDRVTITCRANQTIRTSLNWYQQRPGKAPNLLIYGASRLH
SGVPSRFSGGISGADFTLTISSLQPEDFATYYCQQTYGYSRTFGQGTKVDIKR
(SEQ ID NO: 154).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone
SH12 is

AELTQSPFSLSASVGDRVTITCRASHNIYRSLNWFQHKPGEAPKLLVYAASSLQ

RGVPTRFSGSGSGTDFTLTISSLQPEDSATYFCQQSVTFPYTFGQGTKLEIRR

(SEQ ID NO: 155).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone
SH13 is

AELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLRS

GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPYTFGQGTKLEIKR

(SEQ ID NO: 156).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone
SH14 is

AELTQSPSSLSASVGDRVTITCRASQNIRRSLNWYQHKPGRAPRLLIYAASTLQ

SGVPSRFRGSGSGTDFTLTINSLQPADFATYYCQQSSNTPWTFGHGTKVEIKR

(SEQ ID NO: 157).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone
SH16 is

AELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQS

GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPTFGGGTKVEIKR

(SEQ ID NO: 158).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone
SH18 is

AELTQSPSSLSASVGDRVTITCRASQSISIALNWYQQRPGKAPKLLMYATSTLQ

SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYYNKPTFGPGTKVDIKR

(SEQ ID NO: 159).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone
SH20 is

AELTQSPFSLSASVGDRVTITCRASQSISRSLNWYQHKPGEAPKLLIYAASSLQR

GVPPRFSGSGSGTDFTLTISSLQPEDFATYFCQQSVRIPYSFGQGTKLEIKR (SEQ

ID NO: 160).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone SH21 is

AELTQSPSFLSASVGDRVTITCRASQGIRSYLAWYQQKPGKAPKLLIYAASTLQ

SGVPSRFSGSGSGTEFTLTIASLQPDDFATYYCQQLNNYPPFTFGPGTKVDIKR

5 (SEQ ID NO: 161).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone SH24 is

AELTQSPSSLSASVGDRVTITCRASQSISTYLNWYQQRPGKAPNLLIYAASTLQ

RGVPSRFTGSGSGTDFTLTISSLQPEDFATYYCQQSYTTLWTFGQGTKMEIRR

10 (SEQ ID NO: 162).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone SH26 is

AELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQS

GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSFRRYSFGQGTKLEIKR

15 (SEQ ID NO: 163).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone SH28 is

AELTQSPSSLSASVGDRVTITCRADQNIRRSLNWFQQKPGKAPKLLIYAASSLQ

SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSSSTPWTFGRGTKVEIKR

20 (SEQ ID NO: 164).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone SH30 is

AELTQSPSSLSASVGDRVTITCRASQSIRRSLNWYQQSPGKTPKLLIYAASSLQS

GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTLTFGGGTKVEIKR (SEQ

25 ID NO: 165).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone SH32 is

AELTQEPSLTVSPGGTVTLTCASSTGAVTSRYFPNWFQQKPGQAPRALIYGSNN

KHSWTPARFSGSLLGGKAALTLSGVQPEDEAEYYCLLFYAGAWAFGGGTKLT
VL (SEQ ID NO: 166).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone
SH34 is

5   AELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASGLQ
SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPYTFGQGTKLEIKR
(SEQ ID NO: 167).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone
SH36 is

10   AELTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKSPKLLIYAASSLQS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPPAFGPGTKVDIKR
(SEQ ID NO: 168).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone
SH39 is

15   AELTQSPSSLSASVGDRVTITCRASQTIGRYLNWYQQRPGKAPKLLVYAVSSLQ
SGAPSRFSGSGSGTHFTLTITSLQPEDFATYFCQQSYSSPFTFGQGTKVEIKR
(SEQ ID NO: 169).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone
SH41 is

20   AELTQSPSSLSASVGDRVTITCRASQNIRRSLNWYQHKPGRAPRLLIYAASTLQ
SGVPSRFRGSGSGTDFTLTINSLQPADFATYYCQQSSNTPWTFGHGTKVEIKR
(SEQ ID NO: 170).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone
SH44 is

25   AELTQSPSSLSASVGDRVIITCRASQTIPRFLNWYQQKPGKAPVLLIHSISSLQSG
VPSRFSASGSGTEFTLTISSLQPEDFATYYCQQSYSNLSFGPGTTVDIRR (SEQ ID
NO: 171).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone
SH46 is

AELTQSPSSLSASVGDRVTITCRASQYISSYLNWYQQKPGKAPNLLIYAASSLQ
SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQTYSSPSTFGPGTKVDIKR
(SEQ ID NO: 172).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone
SH47 is
AELTQSPSSLSASVGDRVTITCRASQSISNYLNWYQQKPGKAPNLLIYAASSLQ
SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSYPRTFGQGTKVEIRR
(SEQ ID NO: 173).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone
SH48 is
AELTQSPSSLSASVGDRVTITCRASQYISSYLNWYQQKPGKAPNLLIYAASSLQ
SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQTYSSPSTFGPGTKVDIKR
(SEQ ID NO: 174).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone
SH49 is
AELTQSPSSLSASVGDRVTVTCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQ
SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPWTFGQGTKVEIKR
(SEQ ID NO: 175).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone
SH50 is
AELTQSPSSLSASVGDRVTVTCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQ
SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPWTFGQGTKVEIKR
(SEQ ID NO: 176).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone
SH51 is
AELTQSPSFLSASVGDRVTITCRASQGIRSYLAWYQQKPGKAPKLLIYAASTLQ
SGVPSRFSGSGSGTEFTLTISSLQPEDFATYYCQQLNNYPPFTFGPGTKVDIKR
(SEQ ID NO: 177).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone SH52 is

AELTQSPGTLSLSPGERATLSCRASQSISSSYLAWYQQKPGQAPRLLIYGASSRA
TGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIKR

5   (SEQ ID NO: 178).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone SH54 is

AELTQSPSSMSASVGDRVTITCRASQSIGTYLNWYQQKPGKAPKLLIYAASSLQ
SGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPWTFGQGTKVEIKR

10   (SEQ ID NO: 179).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone SH55 is

AELTQPPSASGTPGQRVTISCSGSSSNIGSKYVYWYQQLPGTAPKLLIYSNNQR
PSGVPDRFSAFKSGTSASLAITGLQAEDEANYYCQSYDSGLSGWVFGGGTKLT

15   VL (SEQ ID NO: 180).

The amino acid sequence of the light chain of anti-Rh(D) antibody clone SH56 is

AELTQSPSSLSASVGDRVTITCRASQSISRYLNWYQQKPGKAPKLLIYAASSLQ
SGVPSRFSGSGSGTDFALTISSLLPEDFATYYCQQGYSTPPYSFGQGTKLEIKR

20   (SEQ ID NO: 181).

Nucleotide Sequences of Anti-Rh(D) Heavy and Light Chains

The nucleotide sequences encoding various anti-Rh(D) antibody clone chains were as follows.

The nucleotide sequence encoding the heavy chain of anti-Rh(D)

25   antibody clone SH10 is

GAGGTGCAGCTGCTCGAGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAG
GTCCCTGAGACTCTCCTGTGCAGCGTCTGGGTTCACCTTCAGTAGGAATGG
CATGCACTGGGTCCGCCAGGCTCCTGGCAAGGGGCTGGAGTGGGTGGCATT
TATATGGTTTGATGGAAGTAATAAATACTATGCAGACTCCGTGAAGGGCCG

ATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAA

CAGCCTGAGAGCCGACGACACGGCTGTGTATTACTGTGCGAGAGAGGAGG

CTCTGTTTCGGGGACTTACTCGGTGGTCCTACGGCATGGACGTCTGGGGCC

AAGGGACCACGGTCAGCGTCTCCTCA (SEQ ID NO: 182).

5   The nucleotide sequence encoding the heavy chain of anti-Rh(D)

antibody clone SH16 is

GAGGTGCAGCTGCTCGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTC

CCTGAGACTCTCCTGTGCAGCGTCTGGGTTCACCTTCAGTAGCTATGGCATG

CACTGGGTCCGCCAGGCTCCAGGCAGGGGGCTGGAGTGGGTGGCTCTTATA

10   TGGTACGATGGAGGTAACAAAGAGTATGCAGACTCCGTGAAGGGCCGCTT

CAGCATCTCCAGAGACAACTCCAAGAACACTCTGTATCTGCAAGTGAACAG

CCTGAGAGCCGACGACACGGCTGTCTATTACTGTGCGAGAGACCAGAGAG

CAGCAGCGGGTATCTTTTATTATTCCCGTATGGACGTCTGGGGCCAAGGGA

CCACGGTCACCGTCTCCTCA (SEQ ID NO: 183).

15   The nucleotide sequence encoding the heavy chain of anti-Rh(D)

antibody clone SH17 is

GAGGTGCAGCTGCTCGAGTCTGGGGGAGGCTTGGTCCAGCCGGGGGGGGTC

CCTGAGACTCTCCTGTGGTGCCTCTGGAATCCCCTTTGTTTCCTCTTGGATG

GCCTGGGTCCGCCAGGCCCCAGGGAAGGGGCTGGAGTGGGTGGCCAACAT

20   AAAACAAGATGGAAGTAAGAAAAACTATGTGGACTCTGTGGAGGGCCGAT

TCACCATCTCCAGAGACAACGCGAAGAACTCACTTTATCTGCAAATGGACA

GCCTGAGAGCCGAGGACACGCGGATATATTACTGTGCGCGAGATTCACTTA

CTTGTTTTGACTACTGGGGCCAGGGAGCCCTGGTCACCGTCTCCTCA (SEQ

ID NO: 184).

25   The nucleotide sequence encoding the heavy chain of anti-Rh(D)

antibody clone SH18 is

GAGGTGCAGCTGCTCGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTC

CCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGGAGCTATGCTATG

CACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGCTAC

AGCATATGATGGAAAAAATAAATACTACGCAGACTCCGTGAAGGGCCGAT

TCACCATCTCCAGAGACAATTCCATGAACACGCTGTTTCTGCAAATGAACA

GCCTGAGAGCTGAGGACACGGCTGTGTTTTACTGTGCGAGAGGCGGATTTT

ACTATGATAGTAGTGGTTATTACGGCTTGAGGCACTACTTTGACTCCTGGG

5   GCCAGGGAACCCTGGTCACCGTCTCCTCA (SEQ ID NO: 185).

The nucleotide sequence encoding the heavy chain of anti-Rh(D)

antibody clone SH20 is

GAGGTGCAGCTGCTCGAGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAG

GTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGAAGTTATGCT

10   ATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCGGT

TATATCATATGATGGAAGTACTATATACTACGCAGACTCCGTGAAGGGCCG

ATTCACCATCTCCAGAGCCAATTCCAAGAACACGCTGTTTCTGCAAATGAA

CAGCCTCAGAACTGAGGACACGGCTGTATATTACTGTACGAGAGGGGGGTT

TTACTATGACAGTAGTGGTTATTACGGGTTGAGGCACTACTTTGACTACTGG

15   GGCCAGGGAACCCTGGTCACCGTCTCTTCA (SEQ ID NO: 186).

The nucleotide sequence encoding the heavy chain of anti-Rh(D)

antibody clone SH24 is

GAGGTGCAGCTGCTCGAGTCGGGGGGAGGCGTGGCCCAGCCTGGGAGGTC

CCTGAGACTCTCCTGTGTAGCGTCTGGATTCAGCCTCAGGAGCTATGGCAT

20   GCACTGGGTCCGCCAGGCTCCTGGCAAGGGGCTGGAGTGGGTGGCAGATA

TATGGTTTGATGGAAGTAATAAAGATTATGCAGACTCCGTGAAGGGCCGAT

TCACCATCTCCAGAGACAATTCCAAGAACACGTTGTATCTTCAAATGAACA

GCCTGAGAGCCGAGGACACGGCTGTGTATTATTGTGCGAGAGATTGGAGG

GTGCGGGCCTTTAGTAGTGGCTGGTTAAGTGCTTTTGATATCTGGGGCCAA

25   GGGACAATGGTCACCGTCTCTTCA (SEQ ID NO: 187).

The nucleotide sequence encoding the heavy chain of anti-Rh(D)

antibody clone SH25 is

GAGGTGCAGCTGCTCGAGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAG

GTCCCTGAGACTCGCCTGTGCAGCGTCTGGATTCAGCTTCAGGAGCTATGG

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CATGCACTGGGTCCGCCAGGCTCCAGGCAGGGGGCTGGAGTGGGTGGCATT

TACATGGTTTGATGGAAGCAATAAATATTATGTAGACTCCGTGAAGGGCCG

ATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGGAAATGAA

CAGCCTGAGAGTCGATGACACGGCTGTATATTACTGTGCGAGAGAGGCGCC

TATGCTTCGCGGAATTAGCAGATACTACTACGCGATGGACGTCTGGGGCCC

AGGGACCACGGTCACCGTCTCCTCA (SEQ ID NO: 188).

The nucleotide sequence encoding the heavy chain of each of anti-Rh(D) antibody clones SH28, SH50, and SH53 is

GAGGTGCAGCTGCTCGAGTCTGGGGGAGGCGGGGTCCAGCCTGGGAGGTC

CCTGCGACTCTCCTGTGCGGCGTCTGGATTCACCTTCAATAGTTATGCCATG

TACTGGGTCCGCCAGCCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGCTATA

TGGTATGATGGAAGTAATAAAGAATATGCAGATTTTGTGAAGGGCCGCTTC

ACCATCTCCAGAGACAATTCCAAGAACACGCTGTCTCTGCAAATGAACAGC

CTGAGAGACGAGGACACGGCTGTGTATTACTGTGCGAGAGAGGCGAATCT

CCTCCGTGGCTGGTCTCGATACTACTACGGTATGGACGTCTGGGGCCAAGG

GACCACGGTCACCGTCTCCTCA (SEQ ID NO: 189).

The nucleotide sequence encoding the heavy chain of anti-Rh(D) antibody clone SH32 is

GAGGTGCAGCTGCTCGAGTCGGGGGGAGGCGTGGTCCAGCCTGGGAGGTC

CCTGAGACTCTCCTGTGAAGCGTCTAAATTCACCCTCTACAATTATGGCATG

CACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCATTTATA

TGGTTTGATGGAAGTAATAAATACTATGAAGACTCCGTGAAGGGCCGATTC

ACCGTCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGC

CTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAACTATCTAAG

AAGGTGGCACTTTCTAGGTATTACTACTATATGGACGTCTGGGGCCAGGGG

ACCACGGTCACTGTCTCGTCA (SEQ ID NO: 190).

The nucleotide sequence encoding the heavy chain of anti-Rh(D) antibody clone SH37 is

GAGGTGCAGCTGCTCGAGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAG

GTCCCTGAGACTCTCCTGTGCAGTGTCTGGATTCACCCTAACTAATTATGGC
ATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCACA
TGTCTGGTATGATGGAAGTAAAACAGAATACGCAGACTCCGTCAAGGGCC
GATTCGCCGTCTCCAGAGACAAATCCAAGAACACACTGTTTCTGCAAATGA
ACAGCCTGACAGCCGAGGACACGGCTATTTATTACTGTGCGAGAGAGAGG
AGAGAGAAAGTCTATATATTGTTCTACTCGTGGCTCGACCGCTGGGGCCAG
GGAACCCTGGTCACCGTCTCCTCA (SEQ ID NO: 191).

The nucleotide sequence encoding the heavy chain of anti-Rh(D) antibody clone SH39 is

GAGGTGCAGCTGCTCGAGCAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAG
GTCCCTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGTAGCTATGGC
ATGCACTGGGTCCGCCAGGCTCCAGGCAAGGGACTGGAGTGGGTGGCAGT
TATATGGTTTGATGGAAGTAATAAGGAATATGCAGACTCCGTGAAGGGCCG
ATTCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTACAAATGAA
CAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGAAGAAG
TGGTTCGGGGAGTTATCTTATGGTCTCGGAAGTTTGACTACTGGGGCCAGG
GAACCCTGGTCACCGTCTCCTCA (SEQ ID NO: 192).

The nucleotide sequence encoding the heavy chain of anti-Rh(D) antibody clone SH44 is

GAGGTGCAGCTGCTCGAGTCGGGGGGGAGGCGTGGCCCAGCCTGGGAGGTC
CCTGAGACTCTCCTGTGTAGCGTCTGGATTCAGCCTCAGGAGCTATGGCAT
GCACTGGGTCCGCCAGGCTCCTGGCAAGGGGCTGGAGTGGGTGGCAGATA
TATGGTTTGATGGAAGTAATAAAGATTATGCAGACTCCGTGAAGGGCCGAT
TCACCATCTCCAGAGACAATTCCAAGAACACGTTGTATCTTCAAATGAACA
GCCTGAGAGCCGAGGATACGGCTGTGTATTATTGTGCGAGAGATTGGAGGG
TGCGGGCCTTTAGTAGTGGCTGGTTAAGTGCTTTTGATATCTGGGGCCAAG
GGACAATGGTCACCGTCTCTTCA (SEQ ID NO: 193).

The nucleotide sequence encoding the heavy chain of anti-Rh(D) antibody clone SH47 is

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GAGGTGCAGCTGCTCGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTC
CCTGCGACTCTCTTGTGCAGCCTCTGGATTCAGCTTCAGTAACTATGCTATG
CACTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTAC
ATCATTTGATGGAAGCATTAAAGACTACGCAGACTCCGTGAAGGGCCGATT
CACCATCTCCAGAGACAATTCCAAGAACACACTATATCTGCAAATGAACAG
CCTGAGAGATGAGGACACGGCTGTATATTACTGTGCGAGAGAGCGGGGGA
TGATAGTCGTGGTCCGTCGCAGAAATGCTTTTGATATTTGGGGCCAAGGGA
CAATGGTCACCGTCTCTTCA (SEQ ID NO: 194).

The nucleotide sequence encoding the heavy chain of anti-Rh(D)
antibody clone SH54 is

GAGGTGCAGCTGCTCGAGTCGGGGGGAGGCGTGGTCCAGCCTGGGAGGTC
CCTGAGACTCTCCTGTGCAGCGTCTGGGTTCACCTTCAGTAGGAATGGCAT
GCACTGGGTCCGCCAGGCTCCTGGCAAGGGGCTGGAGTGGGTGGCATTTAT
ATGGTTTGATGGAAGTAATAAATACTATGCAGACTCCGTGAAGGGCCGATT
CACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAG
CCTGAGAGCCGACGACACGGCTGTGTATTACTGTGCGAGAGAGGAGGCTCT
GTTTCGGGGACTTACTCGGTGGTCCTACGGTATGGACGTCTGGGGCCAAGG
GACCACGGTCAGCGTCTCCTCA (SEQ ID NO: 195).

The nucleotide sequence encoding the heavy chain of anti-Rh(D)
antibody clone SH56 is

GAGGTGCAGCTGCTCGAGTCGGGGGGAGGCGTGGTCCAGCCTGGGAGGTC
CCTGAGACTCTCCTGTGCAGCGTCTGGATTCACCTTCAGTAGCTATGGCATG
CACTGGGTCCGGCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAGTTGT
CTACTATGATGGAAGTAACAAACACTATTCAGACTCCGTGAAGGGCCGATT
CACCATCTTCAGAGACAACTCCAAGAACACGCTGTATCTACAAATGGACAG
CCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGAAAGAAATTT
TCGGAGTGGTTATTCCCGCTACTACTACGGTATGGACGTCTGGGGCCCAGG
GACCACGGTCACCGTCTCCTCA (SEQ ID NO: 196).

The nucleotide sequence encoding the light chain of anti-Rh(D) antibody clone SH8 is

GCCGAGCTCACCCAGTCTCCATCCTCCCTGGCTGCGTCTGTCGGAGACAGA
GTCACCATCACTTGCCGGGCAAATCAGACCATCAGAACCTCTTTAAATTGG
TATCAACAAAGACCTGGGAAAGCCCCTAACCTCCTGATCTATGGTGCATCC
AGGTTGCATAGTGGGGTCCCATCAAGGTTTAGTGGCGGTATTTCTGGGGCA
GACTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT
ACTGTCAGCAGACTTACGGTTATTCTCGAACGTTCGGCCAAGGGACCAAGG
TGGATATCAAACGA (SEQ ID NO: 197).

The nucleotide sequence encoding the light chain of anti-Rh(D) antibody clone SH12 is

GCCGAGCTCACCCAGTCTCCATTCTCCCTGTCTGCATCTGTAGGAGACAGA
GTCACCATAACTTGCCGGGCAAGTCACAACATTTACAGGTCTTTAAATTGG
TTTCAGCATAAACCAGGGGAAGCCCCTAAGCTCCTGGTCTATGCTGCATCC
AGTCTGCAGCGTGGGGTCCCAACCAGGTTCAGTGGCAGTGGATCTGGGACA
GATTTCACTCTCACCATCAGCAGTCTTCAACCTGAAGACTCTGCGACTTACT
TCTGTCAACAGAGTGTCACATTCCCCTACACTTTTGGCCAGGGGACCAAGC
TGGAGATCAGACGA (SEQ ID NO: 198).

The nucleotide sequence encoding the light chain of anti-Rh(D) antibody clone SH13 is

GCCGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA
GTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGG
TATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCC
AGTTTGCGAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA
GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT
ACTGTCAACAGAGTTACAGTACCCCCTACACTTTTGGCCAGGGGACCAAGC
TGGAGATCAAACGA (SEQ ID NO: 199).

The nucleotide sequence encoding the light chain of anti-Rh(D) antibody clone SH14 is

GCCGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA

GTCACCATCACTTGCCGGGCAAGTCAGAACATTAGGAGGTCTTTAAATTGG

TATCAACACAAACCAGGGAGAGCCCCTAGACTCCTGATCTATGCTGCATCC

ACTTTGCAAAGTGGGGTCCCATCAAGGTTCAGGGGCAGTGGATCTGGGACA

5   GATTTCACTCTCACCATCAACAGTCTGCAACCTGCAGATTTTGCAACTTACT

ACTGTCAGCAGAGTTCCAATACCCCGTGGACGTTCGGCCATGGGACCAAGG

TGGAAATCAAACGA (SEQ ID NO: 200).

The nucleotide sequence encoding the light chain of anti-Rh(D)

antibody clone SH16 is

10   GCCGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCCTCTGTAGGAGACAGA

GTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGG

TATCAACAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCC

AGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA

GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT

15   ACTGTCAACAGAGTTACAGTACCCCTCCAACTTTCGGCGGAGGGACCAAGG

TGGAGATCAAACGA (SEQ ID NO: 201).

The nucleotide sequence encoding the light chain of anti-Rh(D)

antibody clone SH18 is

GCCGAGCTCACCCAGTCTCCATCCTCCCTCTCTGCATCTGTAGGAGACAGA

20   GTCACCATCACTTGCCGGGCAAGTCAGAGTATTAGCATCGCTTTAAATTGG

TATCAGCAGAGACCAGGGAAAGCCCCTAAGCTCCTGATGTATGCTACATCC

ACTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA

GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT

ACTGTCAACAATATTACAATAAACCTACTTTCGGCCCTGGGACCAAGGTGG

25   ATATCAAACGA (SEQ ID NO: 202).

The nucleotide sequence encoding the light chain of anti-Rh(D)

antibody clone SH20 is

GCCGAGCTCACCCAGTCTCCATTCTCCCTGTCTGCATCTGTCGGAGACAGA

GTCACCATAACTTGCCGGGCAAGTCAGAGCATTAGCAGGTCTTTAAATTGG

TATCAACATAAACCAGGGGAAGCCCCTAAGCTCCTGATCTATGCTGCATCC

AGTCTGCAGCGTGGGGTCCCACCCAGGTTCAGTGGCAGTGGATCTGGGACA

GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGACTTTGCGACTTACT

TCTGTCAACAGAGTGTCAGAATCCCGTACAGTTTTGGCCAGGGGACCAAGC

TGGAGATCAAACGA (SEQ ID NO: 203).

The nucleotide sequence encoding the light chain of anti-Rh(D)
antibody clone SH21 is

GCCGAGCTCACCCAGTCTCCATCCTTCCTGTCTGCATCTGTAGGAGACAGA

GTCACCATCACTTGCCGGGCCAGTCAGGGCATTAGGAGTTATTTAGCCTGG

TATCAGCAAAAACCAGGGAAAGCCCCTAAGCTCCTAATCTATGCTGCATCC

ACTTTGCAAAGTGGGGTCCCATCAAGGTTCAGCGGCAGTGGATCTGGGACA

GAATTCACTCTCACAATCGCCAGCCTGCAGCCTGATGATTTTGCAACTTATT

ACTGTCAACAGCTTAATAATTACCCCCCTTTCACTTTCGGCCCTGGGACCAA

AGTGGATATCAAACGA (SEQ ID NO: 204).

The nucleotide sequence encoding the light chain of anti-Rh(D)
antibody clone SH24 is

GCCGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA

GTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCACCTATTTAAATTGG

TATCAGCAGAGACCAGGGAAAGCCCCTAACCTCCTGATCTATGCTGCATCC

ACTTTGCAAAGGGGGGTCCCATCAAGGTTCACTGGCAGTGGATCTGGGACA

GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT

ACTGTCAACAGAGTTACACTACCCTGTGGACGTTCGGCCAAGGGACCAAGA

TGGAAATCAGACGA (SEQ ID NO: 205).

The nucleotide sequence encoding the light chain of anti-Rh(D)
antibody clone SH26 is

GCCGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA

GTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGG

TATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCC

AGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA

GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT

ACTGTCAACAGAGTTACAGTTTCCGAAGGTACAGTTTTGGCCAGGGGACCA

5   AGCTGGAGATCAAACGA (SEQ ID NO: 206).

The nucleotide sequence encoding the light chain of anti-Rh(D)

antibody clone SH28 is

GCCGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA

GTCACCATCACTTGCCGGGCAGATCAGAACATTAGGAGGTCTTTAAATTGG

10   TTTCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCC

AGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA

GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT

ACTGTCAACAGAGTTCCAGTACCCCGTGGACGTTCGGCCGAGGGACCAAGG

TGGAAATCAAACGA (SEQ ID NO: 207).

15   The nucleotide sequence encoding the light chain of anti-Rh(D)

antibody clone SH30 is

GCCGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTTGGAGACAGA

GTCACCATCACTTGCCGGGCAAGTCAGAGCATTCGGAGGTCTTTAAATTGG

TATCAGCAGAGTCCAGGGAAAACCCCTAAGCTCCTGATCTATGCTGCATCC

20   AGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA

GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT

ACTGTCAACAGAGTTACAGTACCCTCACTTTCGGCGGAGGGACCAAGGTGG

AGATCAAACGA (SEQ ID NO: 208).

The nucleotide sequence encoding the light chain of anti-Rh(D)

25   antibody clone SH32 is

GCCGAGCTCACTCAGGAGCCCTCACTGACTGTGTCCCCAGGAGGGACAGTC

ACTCTCACCTGTGCTTCCAGCACTGGAGCAGTCACCAGTCGTTACTTTCCAA

ACTGGTTCCAGCAGAAACCTGGCCAGGCACCCAGGGCACTGATTTATGGTT

CAAACAACAAACACTCCTGGACCCCTGCCCGGTTCTCAGGCTCCCTCCTTG

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GGGGCAAAGCTGCCCTGACACTGTCAGGTGTGCAGCCTGAGGACGAGGCG
GAGTATTACTGCCTGCTCTTCTATGCTGGTGCTTGGGCGTTCGGCGGAGGG
ACCAAGCTGACCGTCCTA (SEQ ID NO: 209).

The nucleotide sequence encoding the light chain of anti-Rh(D)
antibody clone SH34 is
GCCGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA
GTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGG
TATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCC
GGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA
GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT
ACTGTCAACAGAGTTACAGTACCCCCCGTACACTTTTGGCCAGGGGACCA
AGCTGGAGATCAAACGA (SEQ ID NO: 210).

The nucleotide sequence encoding the light chain of anti-Rh(D)
antibody clone SH36 is
GCCGAGCTCACTCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA
GTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGG
TATCAGCAGAAACCAGGGAAATCCCCTAAGCTCCTGATCTATGCTGCATCC
AGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA
GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT
ACTGTCAACAGAGTTACAGTACCCCTCCGGCTTTCGGCCCTGGGACCAAAG
TGGATATCAAACGA (SEQ ID NO: 211).

The nucleotide sequence encoding the light chain of anti-Rh(D)
antibody clone SH39 is
GCCGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTGGGAGACAGA
GTCACCATCACTTGCCGGGCAAGTCAGACCATTGGGAGGTATTTAAATTGG
TATCAGCAGAGGCCAGGGAAAGCCCCCAAACTCCTGGTATATGCTGTGTCC
AGTTTGCAAAGTGGGGCCCCATCAAGGTTCAGTGGCAGTGGCTCTGGGACA
CATTTCACTCTCACCATCACCAGTCTGCAACCTGAAGATTTTGCAACTTACT

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TCTGCCAACAGAGTTACAGTTCTCCTTTCACTTTTGGCCAGGGGACCAAGGT

TGAGATCAAACGA (SEQ ID NO: 212).

The nucleotide sequence encoding the light chain of anti-Rh(D)
antibody clone SH41 is

GCCGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA

GTCACCATCACTTGCCGGGCAAGTCAGAACATTAGGAGGTCTTTAAATTGG

TATCAACACAAACCAGGGAGAGCCCCTAGACTCCTGATCTATGCTGCATCC

ACTTTGCAAAGTGGGGTCCCATCAAGGTTCAGGGGCAGTGGATCTGGGACA

GATTTCACTCTCACCATCAACAGTCTGCAACCTGCAGATTTTGCAACTTACT

ACTGTCAGCAGAGTTCCAATACCCCGTGGACGTTCGGCCATGGGACCAAGG

TGGAAATCAAACGA (SEQ ID NO: 213).

The nucleotide sequence encoding the light chain of anti-Rh(D)
antibody clone SH44 is

GCCGAGCTCACCCAGTCTCCATCGTCCCTGTCTGCATCTGTAGGAGACAGA

GTCATCATCACTTGCCGGGCAAGTCAGACCATTCCCAGGTTCTTGAATTGGT

ATCAACAGAAGCCTGGAAAAGCCCCTGTTCTCCTGATTCATAGTATATCCA

GTTTACAAAGTGGGGTCCCATCAAGGTTCAGTGCCAGTGGATCTGGGACAG

AGTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTCGCAACTTACTA

CTGCCAACAGAGTTACAGTAATCTCTCTTTCGGCCCTGGGACCACAGTGGA

TATTAGACGA (SEQ ID NO: 214).

The nucleotide sequence encoding the light chain of anti-Rh(D)
antibody clone SH46 is

GCCGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA

GTCACCATCACTTGCCGGGCAAGTCAGTACATTAGCAGCTATTTAAATTGG

TATCAGCAGAAACCAGGGAAAGCCCCTAATCTCCTGATCTATGCTGCATCC

AGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA

GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT

ACTGTCAACAGACTTACAGTTCCCCTAGCACTTTCGGCCCTGGGACCAAAG

TGGATATCAAACGA (SEQ ID NO: 215).

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The nucleotide sequence encoding the light chain of anti-Rh(D) antibody clone SH47 is

GCCGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA
GTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAACTATTTAAATTGG
TATCAGCAGAAACCAGGAAAAGCCCCTAACCTCCTGATCTATGCTGCATCC
AGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA
GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT
ACTGTCAACAGAGTTACAGTTATCCTCGCACGTTCGGCCAAGGGACCAAGG
TGGAGATCAGACGA (SEQ ID NO: 216).

The nucleotide sequence encoding the light chain of anti-Rh(D) antibody clone SH48 is

GCCGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA
GTCACCATCACTTGCCGGGCAAGTCAGTACATTAGCAGCTATTTAAATTGG
TATCAGCAGAAACCAGGGAAAGCCCCTAATCTCCTGATCTATGCTGCATCC
AGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA
GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT
ACTGTCAACAGACTTACAGTTCCCCTAGCACTTTCGGCCCTGGGACCAAAG
TGGATATCAAACGA (SEQ ID NO: 217).

The nucleotide sequence encoding the light chain of anti-Rh(D) antibody clone SH49 is

GCCGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA
GTCACCGTCACTTGCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGG
TATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCC
AGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA
GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT
ACTGTCAACAGAGTTACAGTACCCCGTGGACGTTCGGCCAAGGGACCAAG
GTGGAAATCAAACGA (SEQ ID NO: 218).

The nucleotide sequence encoding the light chain of anti-Rh(D) antibody clone SH50 is

GCCGAGCTCACCCAGTCTCCATCGTCCCTGTCTGCATCTGTAGGAGACAGA
GTCACCATCACTTGCCGGACAAGTCAGAGCATTGGCACCTATTTAAATTGG
TATCAACAAAAACCAGGGAAAGCCCCTAAACTCCTGATCTATGCTGCATCC
AATGTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCGGTGGATCTGGGACA

GGTTTCTCTCTCATCATCAGCAGTCTGCAACCTGAAGATTTAGCAATTTACT
ACTGCCAACAGAGCTACAGTGTCCCTCCGTACAGCTTTGGCCCGGGGACCA
AGCTGGAGATCAAACGA (SEQ ID NO: 219).

The nucleotide sequence encoding the light chain of anti-Rh(D)
antibody clone SH51 is

GCCGAGCTCACACAGTCTCCATCCTTCCTGTCTGCATCTGTAGGAGACAGA
GTCACCATCACTTGCCGGGCCAGTCAGGGCATAAGGAGTTATTTAGCCTGG
TATCAGCAAAAACCAGGGAAAGCCCCTAAGCTCCTAATCTATGCTGCATCC
ACTTTGCAAAGTGGGGTCCCATCAAGGTTCAGCGGCAGTGGATCTGGGACA
GAATTCACTCTCACAATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATT

ACTGTCAACAGCTTAATAATTACCCCCCTTTCACTTTCGGCCCTGGGACCAA
AGTGGATATCAAACGA (SEQ ID NO: 220).

The nucleotide sequence encoding the light chain of anti-Rh(D)
antibody clone SH52 is

GCCGAGCTCACACAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGA
GCCACCCTCTCCTGCAGGGCCAGTCAGAGTATTAGCAGCAGCTACTTAGCC
TGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCA
TCCAGCAGGGCCACTGGCATCCCAGACAGATTCAGTGGCAGTGGGTCTGGG
ACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAGTG
TATTACTGTCAGCAGTATGGTAGCTCACCGTGGACGTTCGGCCAAGGGACC

AAGGTGGAAATCAAACGA (SEQ ID NO: 221).

The nucleotide sequence encoding the light chain of anti-Rh(D)
antibody clone SH54 is

GCCGAGCTCACCCAGTCTCCATCCTCCATGTCTGCATCTGTAGGAGACAGA
GTCACCATCACTTGCCGGGCAAGTCAGAGCATTGGCACTTATTTAAATTGG

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TATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGCTGCATCC
AGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA
GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACT
ACTGTCAACAGAGTTACAGTACCCCGTGGACGTTCGGCCAAGGGACCAAG

5   GTGGAAATCAAACGA (SEQ ID NO: 222).

The nucleotide sequence encoding the light chain of anti-Rh(D) antibody clone SH55 is

GCCGAGCTCACGCAGCCGCCCTCAGCGTCTGGGACCCCCGGGCAGAGGGT
CACCATCTCTTGTTCTGGAAGCAGCTCCAACATCGGAAGTAAATATGTATA

10   CTGGTACCAGCAACTCCCAGGAACGGCCCCCAAACTCCTCATTTATAGTAA
TAATCAGCGGCCCTCAGGGGTCCCTGACCGATTCTCTGCCTTCAAGTCTGG
CACCTCAGCCTCCCTGGCCATCACTGGGCTCCAGGCTGAGGATGAGGCTAA
TTATTACTGCCAGTCCTATGACAGCGGCCTGAGTGGCTGGGTGTTCGGCGG
CGGGACCAAGCTGACCGTCCTA (SEQ ID NO: 223).

15   The nucleotide sequence encoding the light chain of anti-Rh(D) antibody clone SH56 is

GCCGAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGA
GTCACCATCACTTGCCGGGCAAGTCAGAGCATTAGCAGGTATTTAAATTGG
TATCAGCAGAAACCAGGGAAAGCCCCCAAGCTCCTGATCTATGCTGCATCC

20   AGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACA
GATTTCGCTCTCACCATCAGCAGTCTGCTACCTGAAGATTTTGCAACTTACT
ACTGTCAACAGGGTTACAGTACCCCTCCGTACAGTTTTGGCCAGGGGACCA
AGCTGGAGATCAAACGA (SEQ ID NO: 224).

The disclosures of each and every patent, patent application and

25   publication cited herein are hereby incorporated herein by reference in their entirety.

While this invention has been disclosed with reference to specific
embodiments, it is apparent that other embodiments and variations of this invention
may be devised by others skilled in the art without departing from the true spirit and

scope of the invention. The appended claims are intended to be construed to include all such embodiments and equivalent variations.